Al-Hussein Bin Talal University
Biology Department
MICROBIOLOGY FOR NURSING STUDENTS
2014\2015

Instructor: Dr. Sulaiman Alnaimat

Microbiology for Nurses is a one-semester course that emphasizes the interaction of microorganisms with humans and the diseases they cause. Students will examine how our immune systems develop and function to protect us from the countless dangers we encounter daily, including microbial pathogens and cancerous human cells. In subsequent sections, students investigate the behavior of a wide variety of microbes that comprise the great diversity of life on Earth, including bacteria, and viruses. Students will learn how these organisms work, their pathogenic mechanisms, and preventative and therapeutic strategies to reduce diseases they cause. In summary, topics include microscopy, survey of various microbes, the immune system, microbial pathogens and mechanisms of disease transmission**.**

Syllabus

Chapter	topic
Chapter One	Introduction and General Bacteriology
Chapter Two	Microorganisms Growth and Cultivating
Chapter Three A	Innate Host Defenses Against Microbial Invasion
Chapter Three B	Adaptive Host Defenses Against Microbial Invasion
Chapter Four	Introduction to infectious disease
Chapter Five	Bacterial Pathogenesis
Chapter Six	Control of Infectious Disease
Chapter Seven A:	Viral Pathogenesis
Chapter Seven B:	Some viral infection examples
Chapter Eight	General mycoses

Exams:
First Exam: **25%**
Second Exam: **25%**
Final exam: **50%**

References
1. **Wessner D. Microbiology (2013)**
2. **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**
3. **Kayser FH. 2005. Medical Microbiology: Georg Thieme Verlag.**

# Microbiology for Nursing students

Chapter One: introduction and General Bacteriology
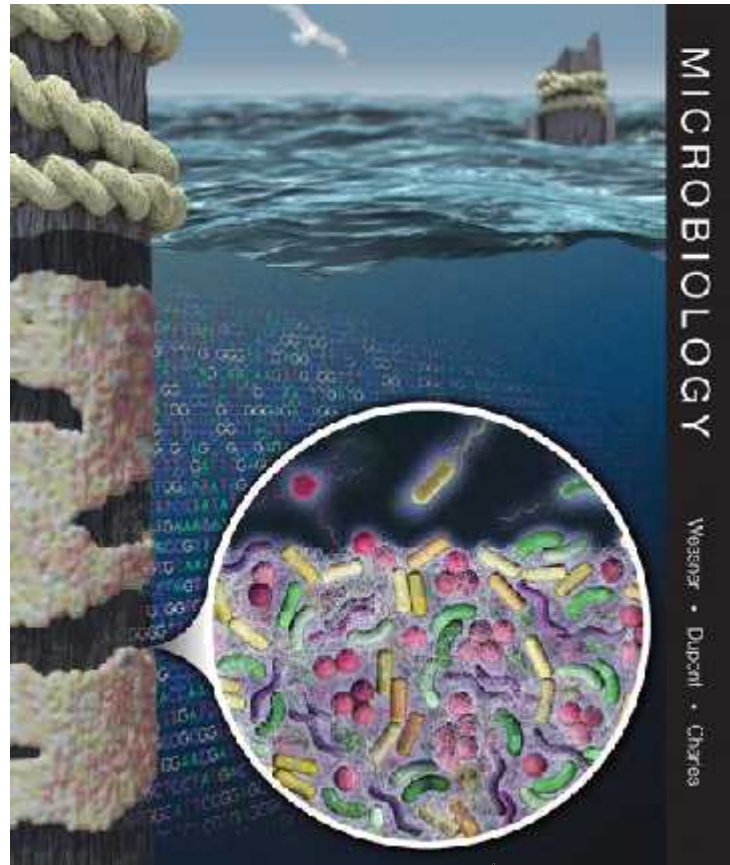
Dr. Sulaiman Alnaimat 2015

Introduction

- *What is microbiology?*
  - Microbiology is the study of microbes.
  - Microbes are forms of life too small to be seen with the naked eye (bacteria, fungi, algae, protists).
  - The field examines how microbes interact with humans, with food, and how they can be used BY humans (among other aspects).

Microbes and disease

Microbes and digestion
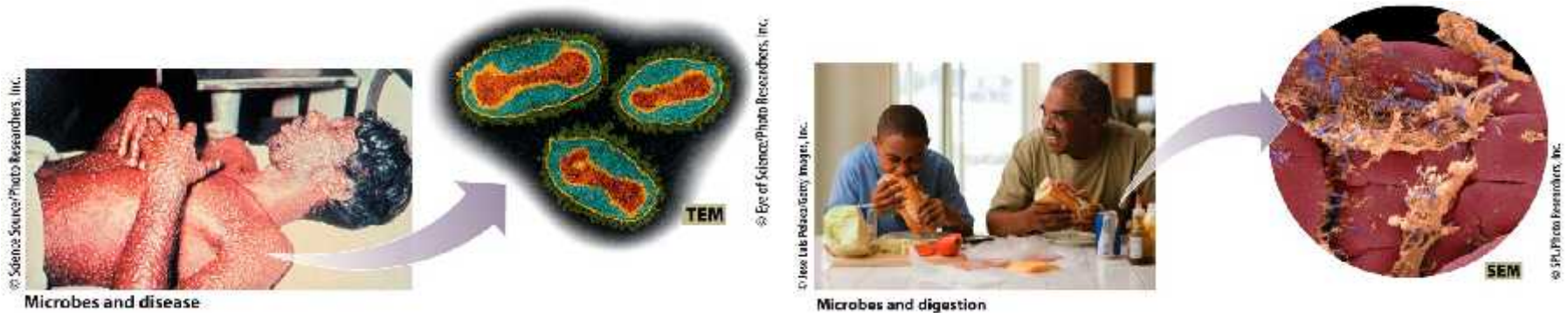
Introduction

- Microbiology is the study of microbes.
  - Microbes are forms of life too small to be seen with the naked eye (bacteria, fungi, algae, protists).
  - The field examines how microbes interact with humans, with food, and how they can be used BY humans (among other aspects)…

A. Some microbes infect important agricultural plants.

B. Other microbes provide nutrients to plants.

C. Many microbes cause food to spoil.

D. Other microbes aid in food and beverage preparation.

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Introduction

- If microbiology is the study of life, what is the basis for life?
  - Metabolism
  - Growth
  - Reproduction
  - Genetic variation/evolution
  - Response/adaptation to the external environment
  - Homeostasis (maintaining internal organization and order, usually by expending energy)

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Introduction

- So if that's life, what macromolecules (major units) are needed for it to happen?

TABLE 1.1 Macromolecules in microbial cells

Macromolecule	Subunits	Functions	Dry weight of cell (%)
Polypeptides	Amino acids	Enzymes catalyze the vast majority of biochemical reactions in the cell. Other proteins are structural components of cells.	50–55
Nucleic acids	Deoxyribonucleotides	Informational: DNA provides the instructions for assembly and reproduction of the cell.	2–5
	Ribonucleotides	Many functions, most of which are involved in the production of polypeptides. Some serve structural or catalytic functions.	15–20
Lipids	Diverse structures	Structural: make up cellular membranes that form physical boundary between the inside of cell and surroundings and membranes of internal organelles.	10
Polysaccharides	Sugars	Structural (such as cellulose and chitin) and energy storage (such as glycogen and starch).	6–7

- What about viruses, though?
  - Technically, viruses aren't considered to be alive.
  - They don't replicate outside of a host cell.
  - They (usually) have little to no biochemical activity outside of a host cell.
  - They are inert and nonreactive outside of a host cell.
  - Microbiology still studies viruses, though, since they are too small to be seen with the naked eye.
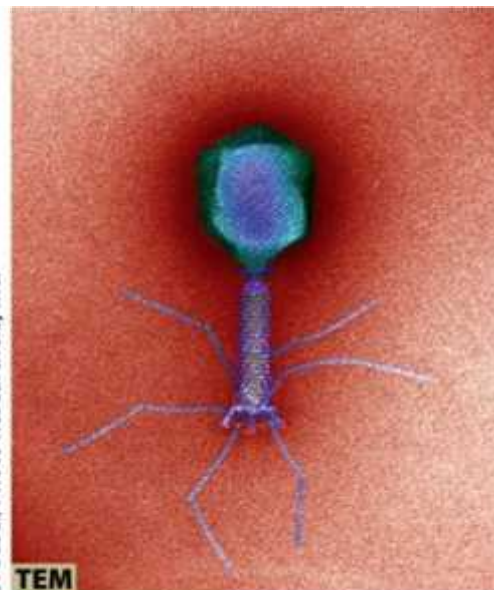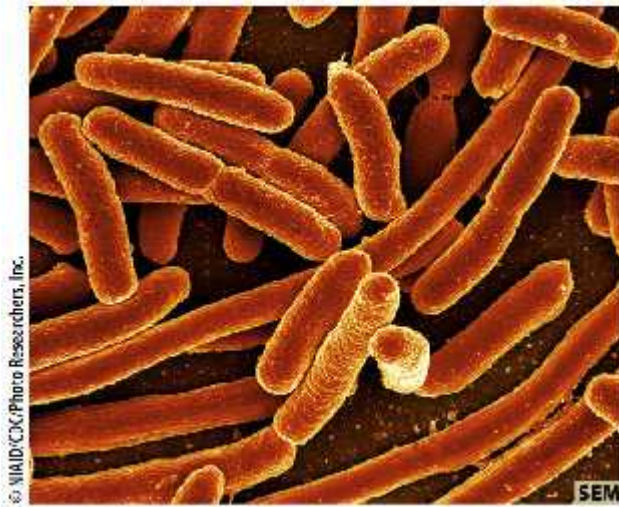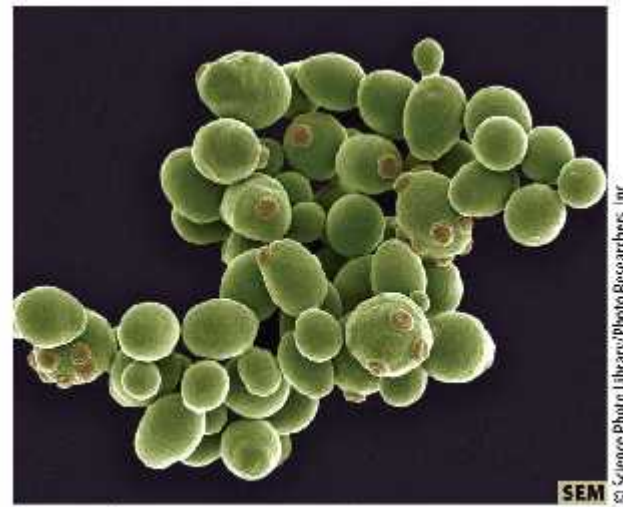

TEM
C. T4 bacteriophage

TEM
B. Ebola virus

6
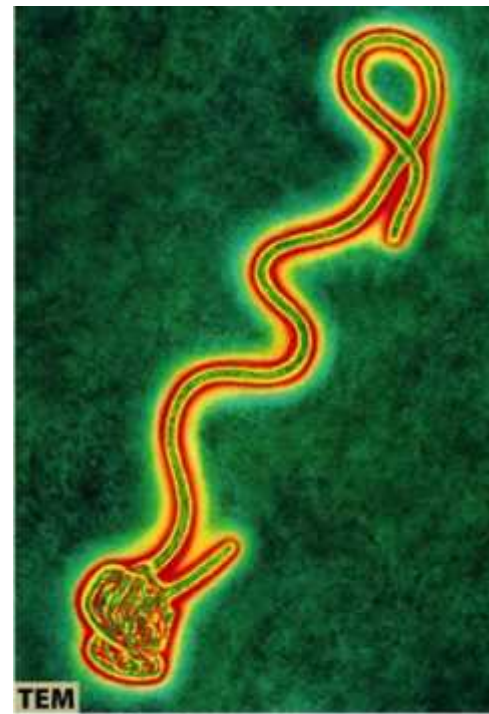
Introduction

A. Escherichia coli   B. Saccharomyces cerevisiae

- So why study microbes at all if they're so hard to see?
  - They're very fast, cheap, and easy to grow.
  - They can produce enzymes and other molecules for industrial/medical uses.
  - Most of them have small numbers of genes, making them simpler to study.
  - Genetic manipulation of single-celled bacteria is usually much easier than multicellular eukarya.
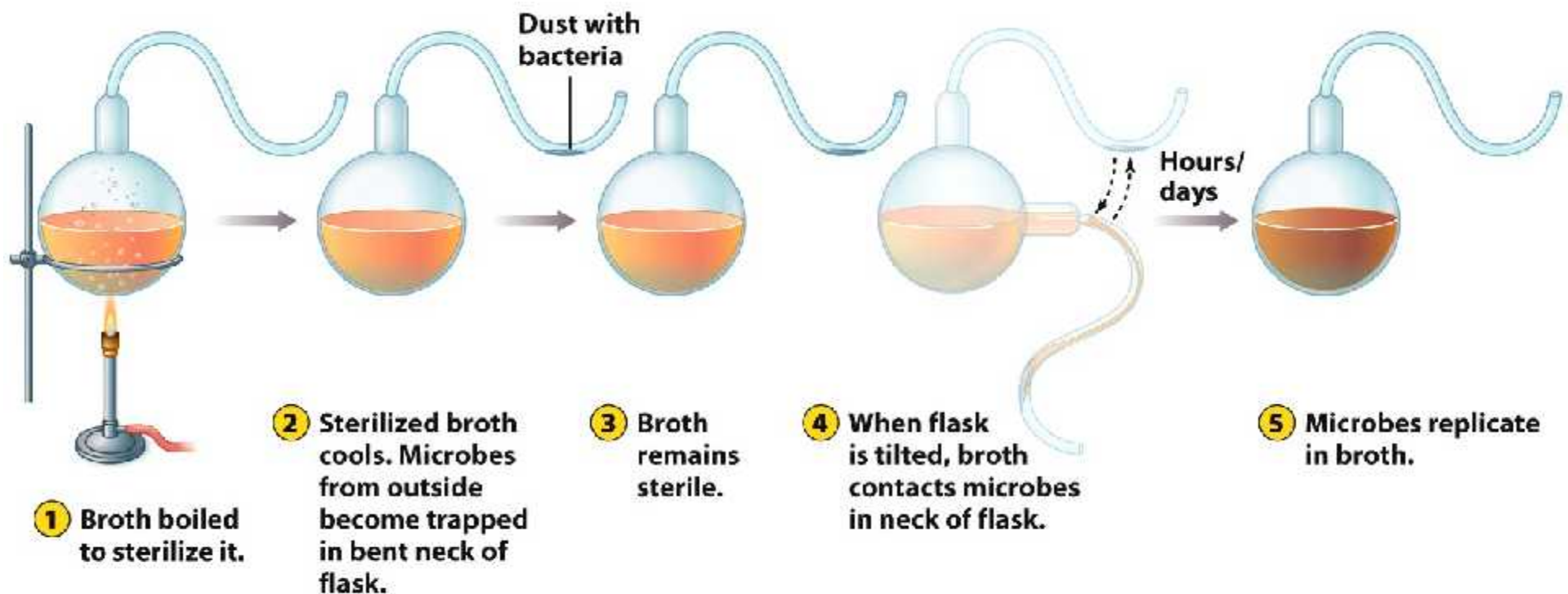
7

Microbial metabolism and ecology:

- BUT be careful! One of the biggest mistakes a student can make when studying microbes is thinking of them as individual cells/populations.

- Microbes live in diverse groups in nature, with many different members forming a microbial community and ecosystem.
  - Microbes in the intestines
  - Plaque on teeth
  - Slime on rocks on beaches
  - Mold growths on bathroom surfaces

Microbes and disease:

- *How are microbes associated with disease?*
  - We didn't always believe that microbes caused disease or existed around us unseen.
  - People used to believe that disease was associated with angry gods or bad air.
  - Even when microbes were known to exist, people thought they could spontaneously form as life from nonliving matter (the spontaneous generation theory).
  - It took the work of many people to debunk these ideas, but two were very important: Louis Pasteur and Robert Koch.

Microbes and disease:

- Louis Pasteur performed a simple yet elegant experiment to disprove spontaneous generation theory in the late 1800s.

Dust with bacteria

Hours/ days

② **Sterilized broth cools. Microbes from outside become trapped in bent neck of flask.**

③ **Broth remains sterile.**

④ **When flask is tilted, broth contacts microbes in neck of flask.**

⑤ **Microbes replicate in broth.**

① **Broth boiled to sterilize it.**

Microbes and disease:

- Robert Koch determined *Bacillus anthracis* and *Mycobacterium tuberculosis* were the causes of anthrax and tuberculosis (respectively).
- His work with anthrax helped sheep herders and cattle ranchers avoid costly animal losses.
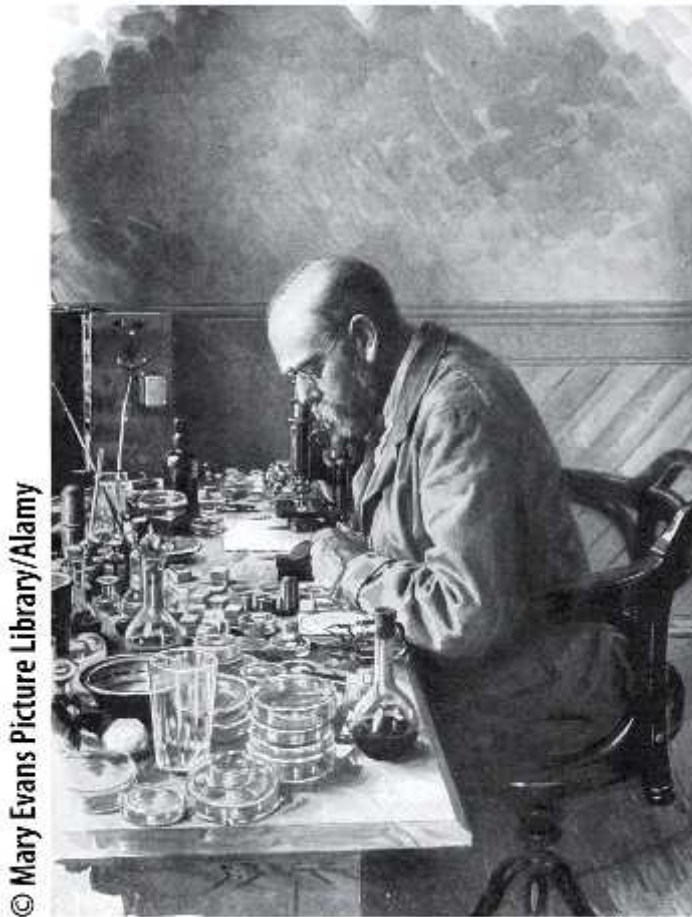

Bacillus anthracis

© Dr. Gary Gaugler/Photo Researchers, Inc.

SEM

Vaccination

Chuck Bigger/© AP/Wide World Photos
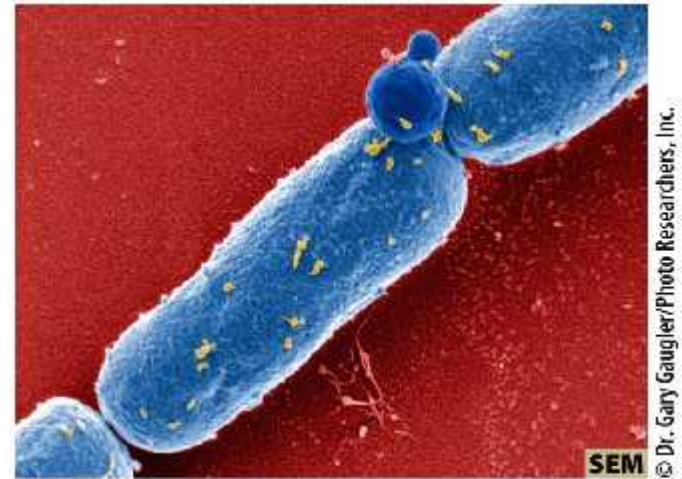
Microbes and disease:

© Mary Evans Picture Library/Alamy

Robert Koch

- The basic rules Koch established made it possible for others to determine which microbes caused which diseases. They are still in use to this day.

- These rules (Koch's postulates) will be discussed in more detail in coming chapters.

- Some microbial diseases have had a profound impact on humanity—plague, for instance.

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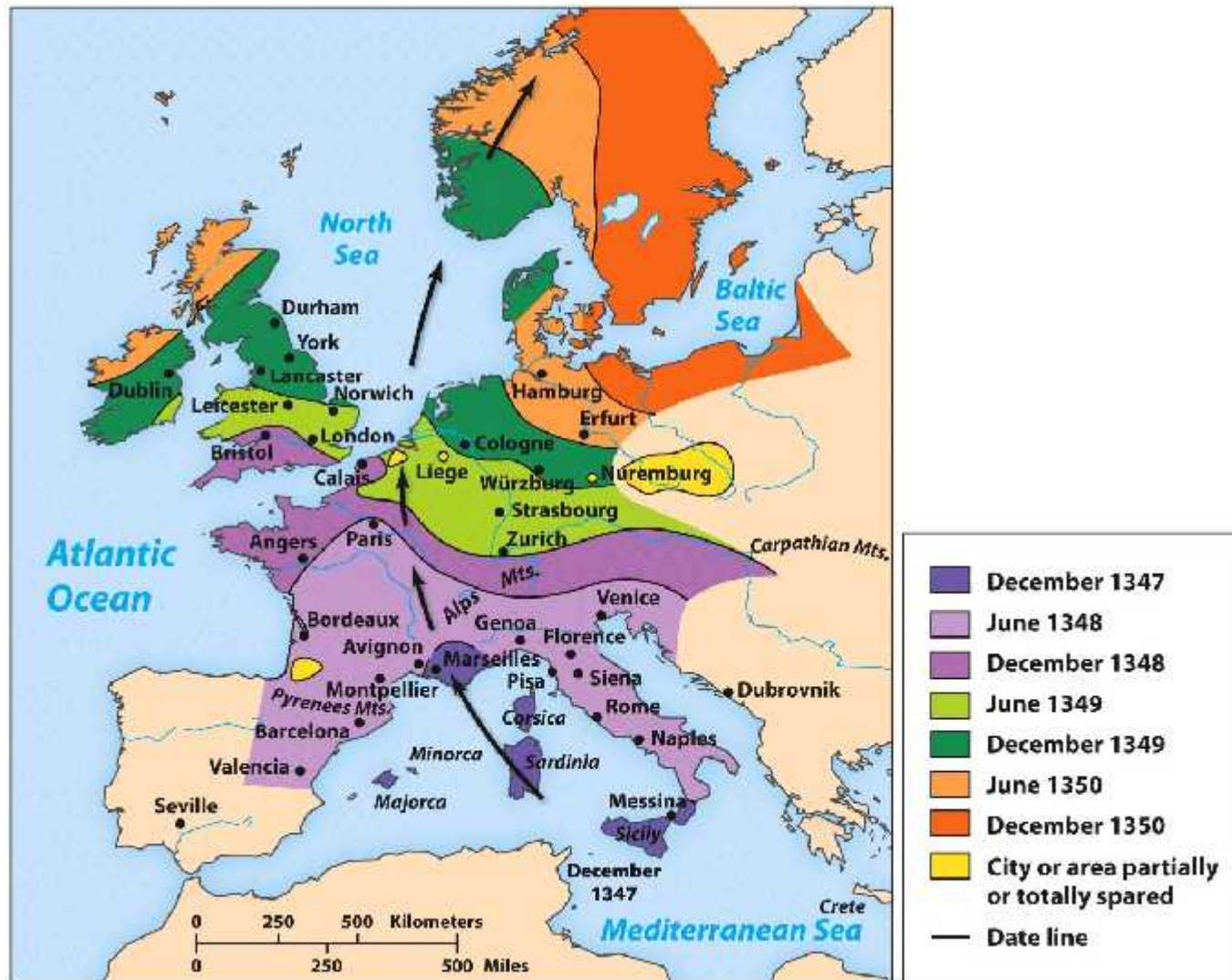
Christian Gautier/Peter Arnold, Inc.

Fleas bite human as alternative host. Flea regurgitates infected rat blood into wound. Bacteria multiply, causing disease and death.

Flea bites rat and feeds on blood. Bacteria multiply in flea gut, which becomes clogged. Flea attempts to feed again.

Kallista Images/Getty Images, Inc.

SEM

Flea bites rat, regurgitates bacteria into wound. New infection starts in rat bloodstream.

mitry Maslov/iStockphoto

Josse Lieferinxe, *Saint Sebastian Interceding for the Plague Stricken*, 1497–1499, Walters Art Museum, Baltimore, USA/Corbis Images

1411 drawing from Torrenburg Bible depicting people infected with *Yersinia pestis* causing plague.

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- In the twentieth century, we have seen a dramatic drop in U.S. deaths from infectious diseases, however…

15
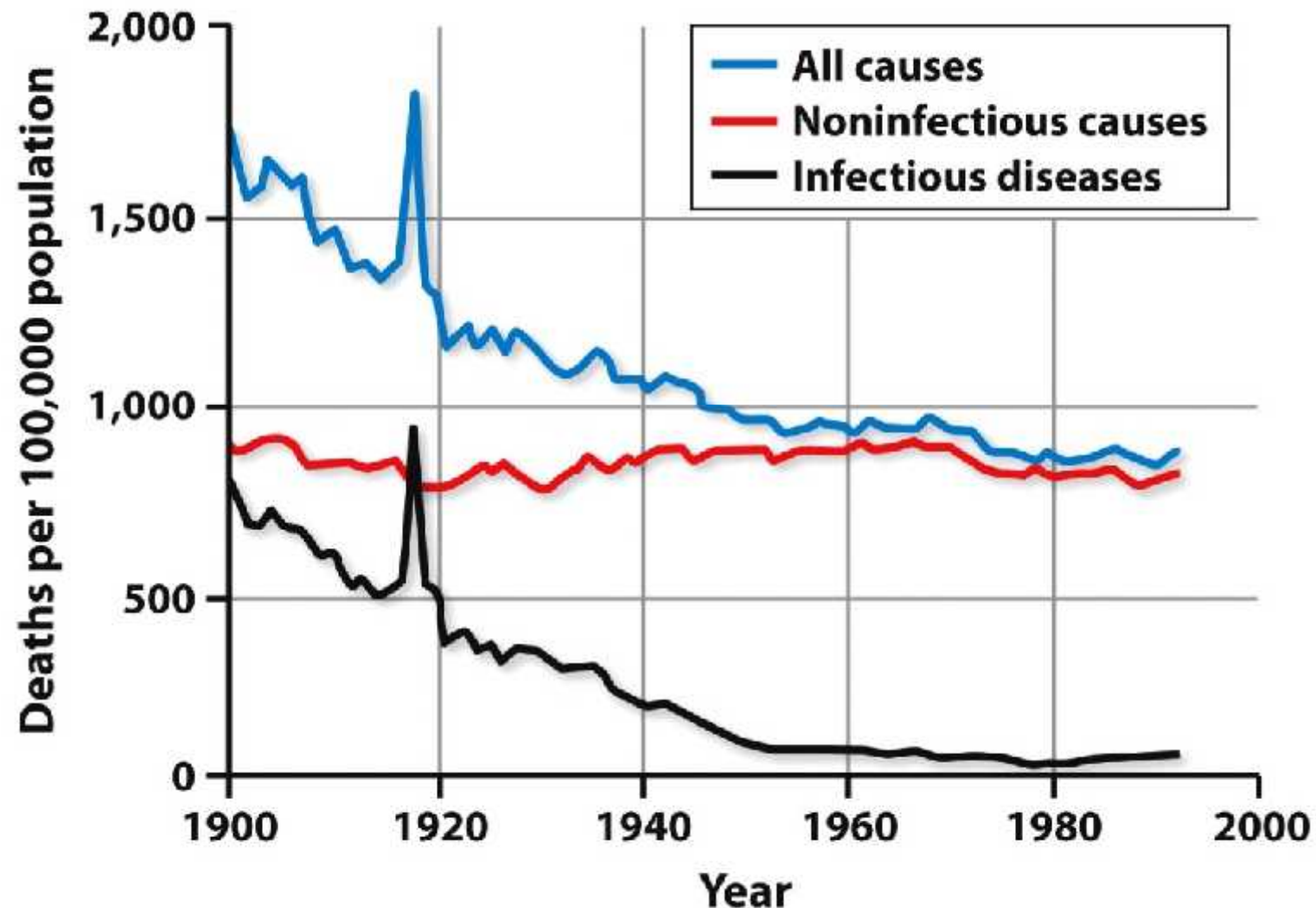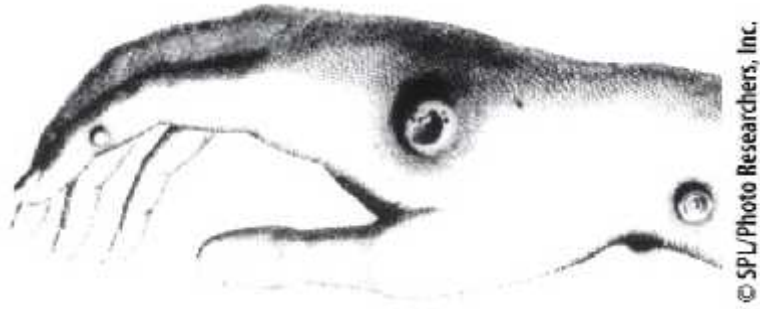
Skin blisters caused by smallpox infection often left permanent scars.

Painting depicting Jenner inoculating a boy against smallpox

Microbes and disease:

- Where has this reduction in deaths come from?
  - Prevention of infection through
    - Use of antiseptics (Joseph Lister)
    - Sanitation improvements (sewage treatment)
    - Food/water safety (pasteurization)
    - Personal hygiene improvements
    - Vaccination
  - Treatment of infections (antibiotics!)

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TABLE 1.4 Selected advances in microbiology

Year	Scientist	Advance
Late 1600s	Anton van Leeuwenhoek	Uses microscope to see microorganisms
1860s	Louis Pasteur	Disproves idea of spontaneous generation
1860s	Joseph Lister	Practices infection control
1876	Robert Koch	Identifies *Bacillus anthracis* as cause of anthrax
1928	Alexander Fleming	Discovers penicillin
1950s	Jonas Salk and Albert Sabin	Develop poliovirus vaccines
1966	Lynn Margulis	Proposes endosymbiotic theory
1983	Kary Mullis	Invents PCR
1990	Carl Woese	Proposes three-domain classification of living organisms
1995	Craig Venter	Publishes first complete bacterial genome sequence

Morphology of bacterial cells:

- Bacteria can take many different shapes (or morphologies).
  - Spherical (*s.* coccus, *pl.* cocci)
  - Rod-shaped (*s.* bacillus, *pl.* bacilli)
  - Comma-shaped (*s.* vibrio*, pl.* vibrios)

© Science Source/Photo Researchers, Inc.

SEM
Staphylococcus aureus

Cocci are spherical   *Streptococcus pyrogenes*
SEM
© Eye of Science/Photo Researchers, Inc.

© Dr. Gary Gaugler/Photo Researchers, Inc.

SEM
Bacillus anthracis

Escherichia coli
SEM
© Dennis Kunkel Microscopy, Inc./Phototake

Bacilli are rod shaped

© Dennis Kunkel Microscopy, Inc./Phototake
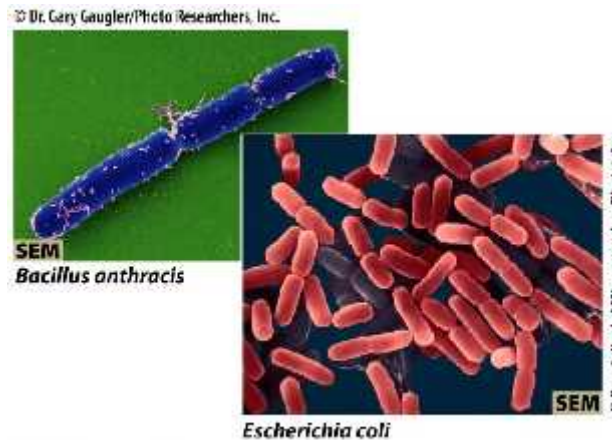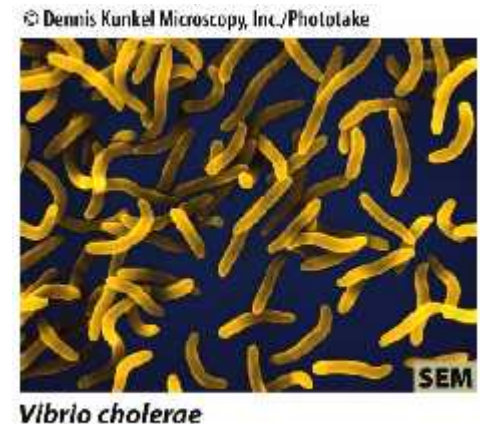
Vibrio cholerae
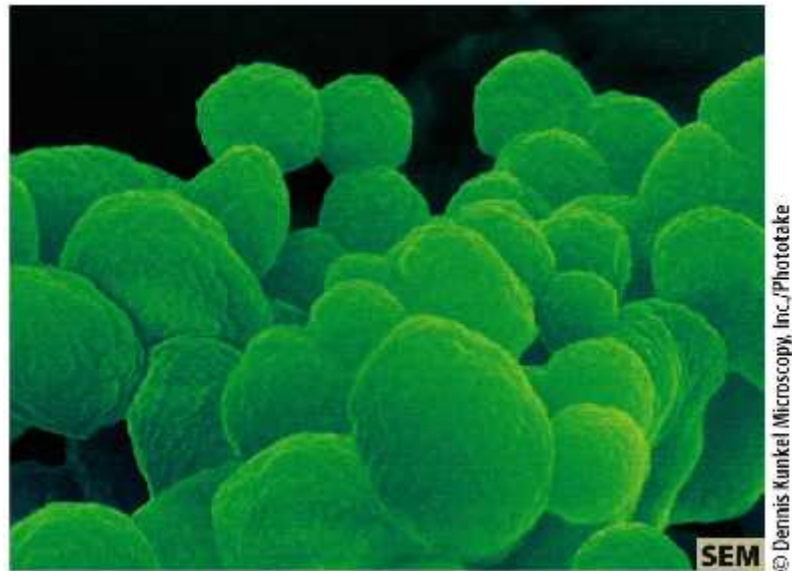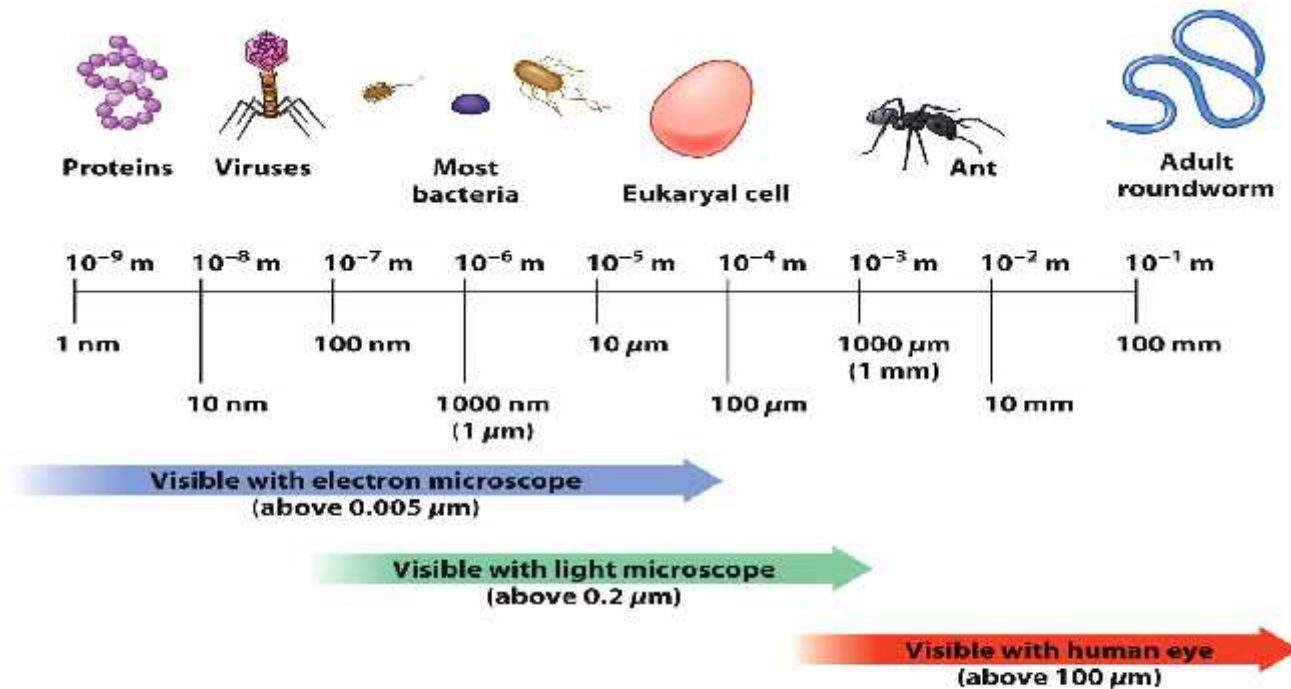SEM
Vibrio are slightly curved rods

Morphology of bacterial cells:

- Bacteria can take many different shapes (or morphologies).
  - Spiral (*s.* spirillum, *pl.* spirilla)
  - Pleiomorphic (varied shapes)

© James Cavallini/Photo Researchers, Inc.

LM

Treponema pallidum
Spirilla are spiral shaped

SEM

© Dennis Kunkel Microscopy, Inc./Phototake

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Morphology of bacterial cells:

Proteins | Viruses | Most bacteria | Eukaryal cell | Ant | Adult roundworm

10^{-9} m 10^{-8} m 10^{-7} m 10^{-6} m 10^{-5} m 10^{-4} m 10^{-3} m 10^{-2} m 10^{-1} m

1 nm   100 nm   10 μm   1000 μm (1 mm)   100 mm

10 nm   1000 nm (1 μm)   100 μm   10 mm

Visible with electron microscope (above 0.005 μm)

Visible with light microscope (above 0.2 μm)

Visible with human eye (above 100 μm)

- Size of bacteria can vary greatly.
  - Usually smaller than eukaryal cells (bacteria are often 0.5–5 μm in length)
  - SMALL eukaryal cells are usually >5 μm in diameter
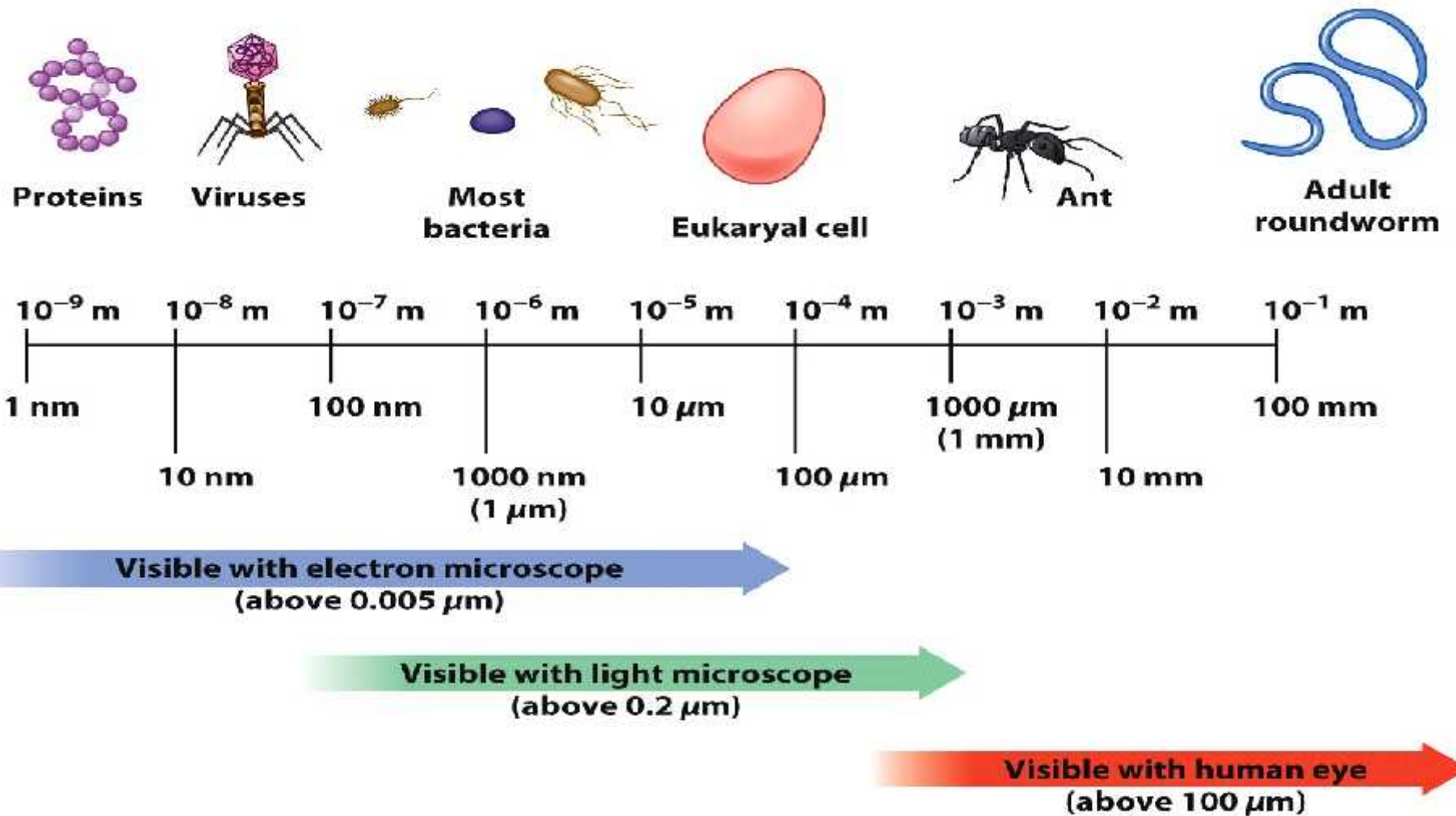
20

Proteins   Viruses   Most bacteria   Eukaryal cell   Ant   Adult roundworm

10^{-9} m 10^{-8} m 10^{-7} m 10^{-6} m 10^{-5} m 10^{-4} m 10^{-3} m 10^{-2} m 10^{-1} m

1 nm   100 nm   10 μm   1000 μm (1 mm)   100 mm

10 nm   1000 nm (1 μm)   100 μm   10 mm

Visible with electron microscope
(above 0.005 μm)

Visible with light microscope
(above 0.2 μm)

Visible with human eye
(above 100 μm)

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The cytoplasm:

- *What is in the cytoplasm of bacterial cells?*
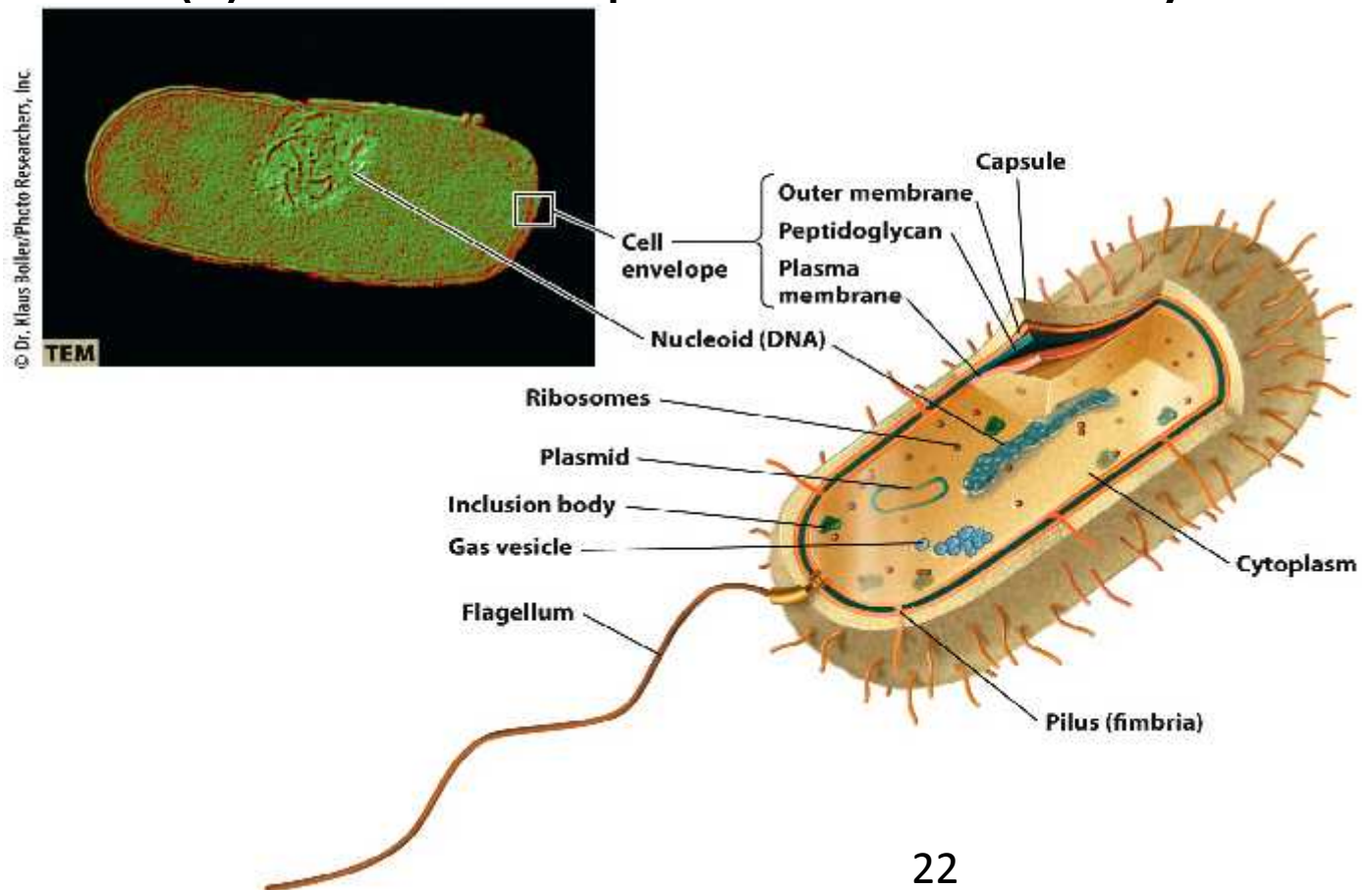 - The largest area is the nucleoid region, housing the chromosome(s) and DNA replication machinery.

© Dr. Klaus Boller/Photo Researchers, Inc.

TEM

Cell envelope
- Outer membrane
- Peptidoglycan
- Plasma membrane

Capsule

Nucleoid (DNA)

Ribosomes

Plasmid

Inclusion body

Gas vesicle

Flagellum

Cytoplasm

Pilus (fimbria)

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TABLE 2.1 Features of the bacterial cytoplasm

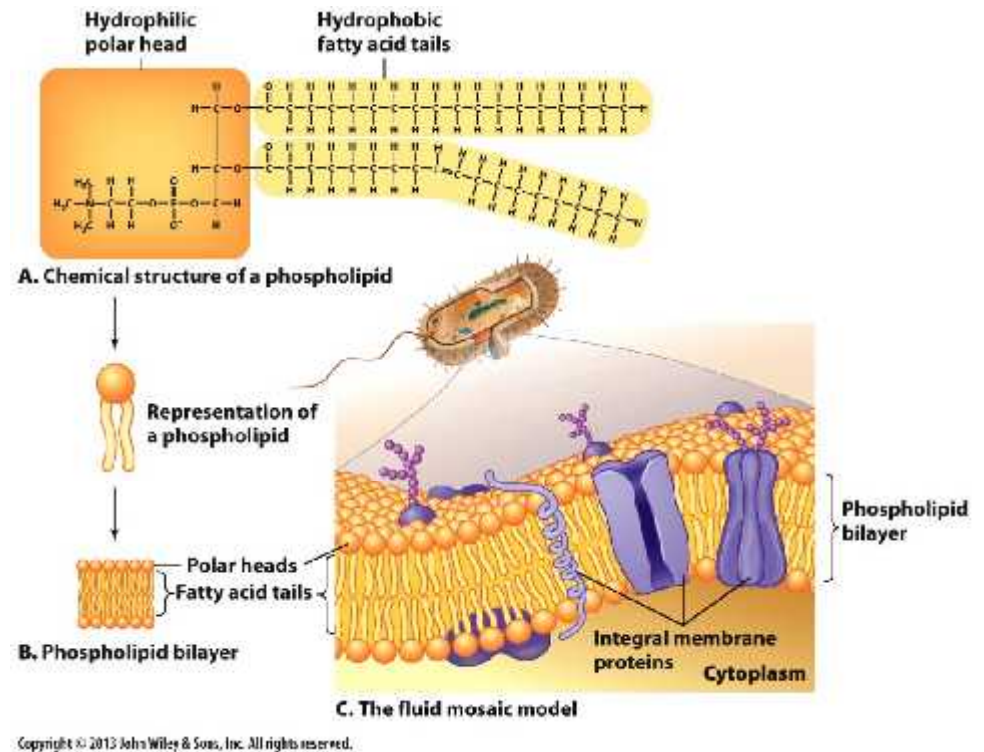| Organelle or molecules	Composition	Function
DNA nucleoid	DNA, RNA, protein	Genetic information storage and gene expression
Chromosome-packaging proteins	Protein	Protection and compaction of genomic DNA
Enzymes involved in synthesis of DNA, RNA	Protein	Replication of the genome, transcription
Regulatory factors	Protein, RNA	Control of replication, transcription, and translation
Ribosomes	RNA, protein	Translation (protein synthesis)
Plasmid(s)	DNA	Variable, encode non-chromosomal genes for a variety of functions
Enzymes involved in breaking down substrates	Protein	Energy production, providing anabolic precursors
Inclusion bodies	Various polymers	Storage of carbon, phosphate, nitrogen, sulfur
Gas vesicles	Protein	Buoyancy
Magnetosomes	Protein, lipid, iron	Orienting cell during movement
Cytoskeletal structures	Protein	Guiding cell wall synthesis, cell division, and possibly partitioning of chromosomes during replication

The cytoplasm:

- *What is in the cytoplasm of bacterial cells?*
  - The remainder of the cytoplasm is a stew of macromolecules (tRNA, rRNA, mRNA, proteins, etc.).
  - Inclusion bodies may also be present.
    - Polyhydroxybutyrate granules: carbon storage
    - Sulfur globules: sulfur storage
    - Gas vesicles: buoyancy control
    - Carboxysomes: location of carbon fixation reactions
    - Magnetosomes: organelle associated with direction finding

The bacterial cytoskeleton:

- *What kinds of internal structures help to organize bacterial cells?*
  - The cytoskeleton is a series of internal proteins that assist in keeping everything in (or moving it to) the right locations in cells.
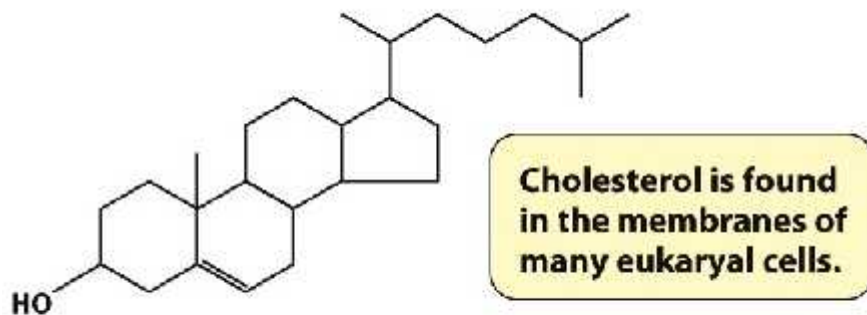
The cell envelope:

- *What are the critical structural and functional properties of the bacterial cell envelope?*
  - ALL cells have a plasma membrane (PM).
    - Separates the interior of the cell from the external environment
    - Usually composed of a phospholipid bilayer with embedded proteins

Hydrophilic polar head

Hydrophobic fatty acid tails

A. Chemical structure of a phospholipid

Representation of a phospholipid

Polar heads
Fatty acid tails

B. Phospholipid bilayer

Integral membrane proteins

Phospholipid bilayer
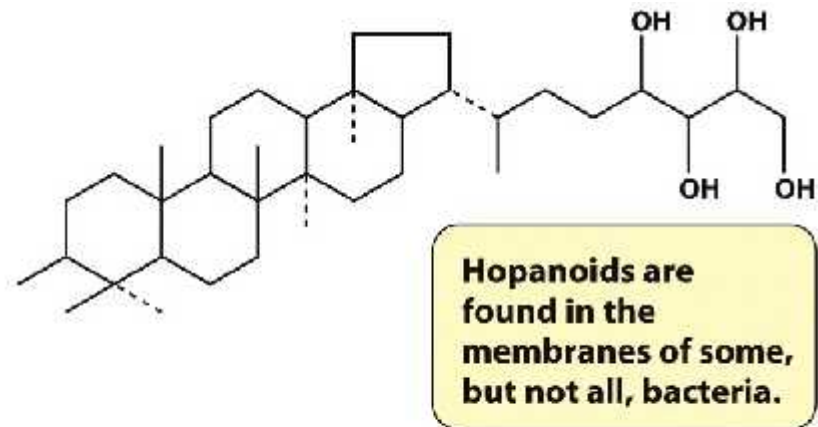
Cytoplasm

C. The fluid mosaic model

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The cell envelope:

- *What are the critical structural and functional properties of the bacterial cell envelope?*
  - The PM may have sterol molecules called "hopanoids" in it to help with stability across temperature ranges.

Cholesterol is found in the membranes of many eukaryal cells.

A. Cholesterol

Hopanoids are found in the membranes of some, but not all, bacteria.

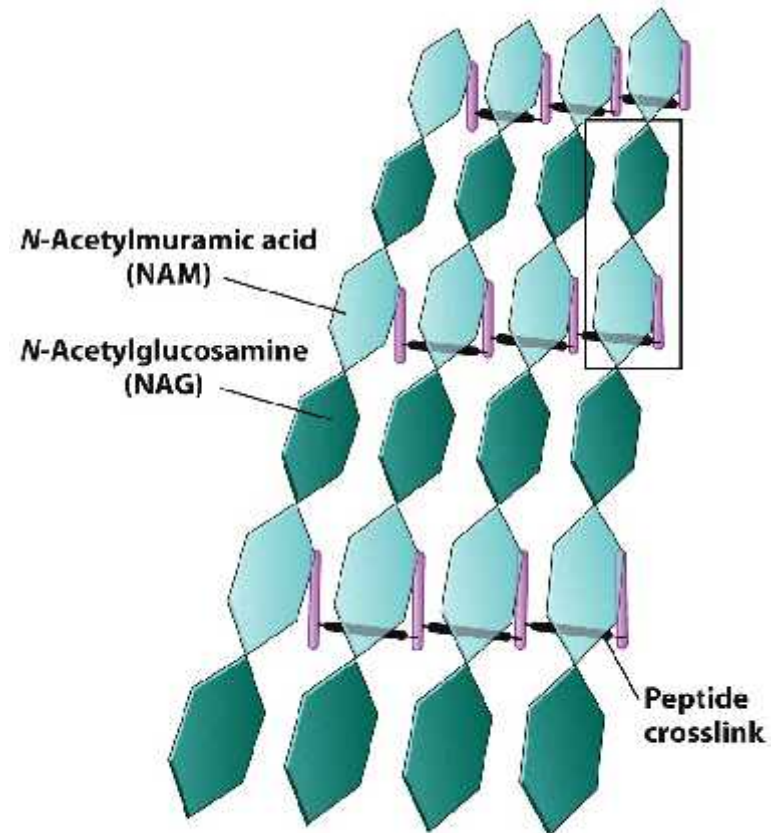B. Bacteriohopanetetrol

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The cell envelope:

- *What are the critical structural and functional properties of the bacterial cell envelope?*
  - How do items cross the PM and get into a cell?
    - O_2 and CO_2 are small and can diffuse across readily.
    - H_2O is helped across by aquaporin protein channels.
    - Osmosis is the flow of water across the PM toward the side with a higher solute (particle) concentration.
    - Osmosis can cause a cell to swell with water or shrivel as water leaves, but a strong cell wall can help keep a bacterial cell alive during these hardships.

The cell envelope:

- *What are the critical structural and functional properties of the bacterial cell envelope?*
  - The PM can also be used for capturing energy.
    - Embedded electron transport chains can help create proton motive force (PMF).
    - Can be used for respiration/photosynthesis (more in Chapters 6 and 13)
    - Can be used to derive energy for motion (flagella)
  - The PM can hold sensory systems.
    - Proteins in the PM can be used to detect environment changes.
    - The cell can use the detected changes to alter gene expression to respond.
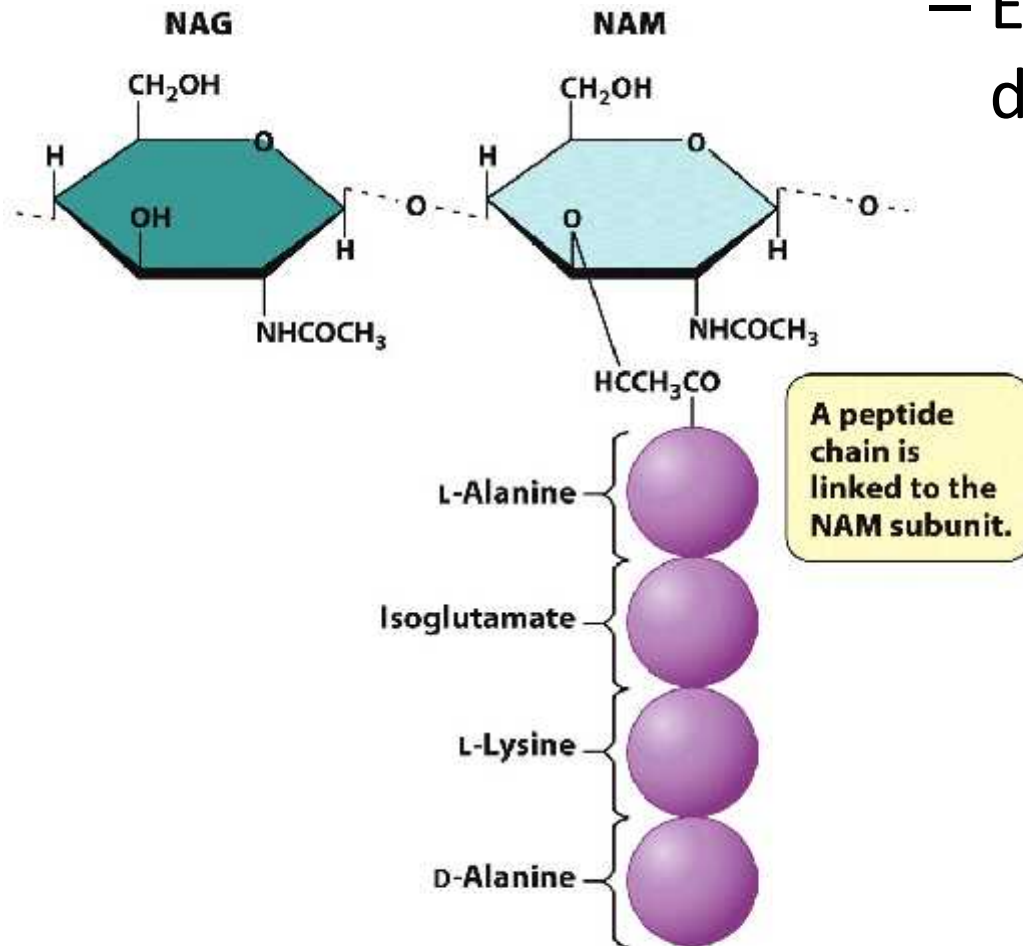
The cell Wall:

– The bacterial cell wall (CW) is a crucial structure.

– It is composed of crosslinked strands of peptidoglycan subunits forming a matrix (similar to a chain-link fence).

– It gives the cells their shape and protection from osmotic lysis/mechanical forces.

N-Acetylmuramic acid (NAM)

N-Acetylglucosamine (NAG)

Peptide crosslink

Crosslinked peptidoglycan

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The cell envelope:

NAG

CH_2OH

H

OH

H

O

O

NHCOCH₃

NAM

CH_2OH

H

O

O

O

H

NHCOCH₃

$HCCH_3CO$

L-Alanine

Isoglutamate

L-Lysine

D-Alanine

A peptide chain is linked to the NAM subunit.

Disaccharide backbone with peptide chain

– Each peptidoglycan disaccharide subunit is
  - *N*-acetylmuramic acid (NAM) with a small peptide chain
    – *The peptide varies by species.*
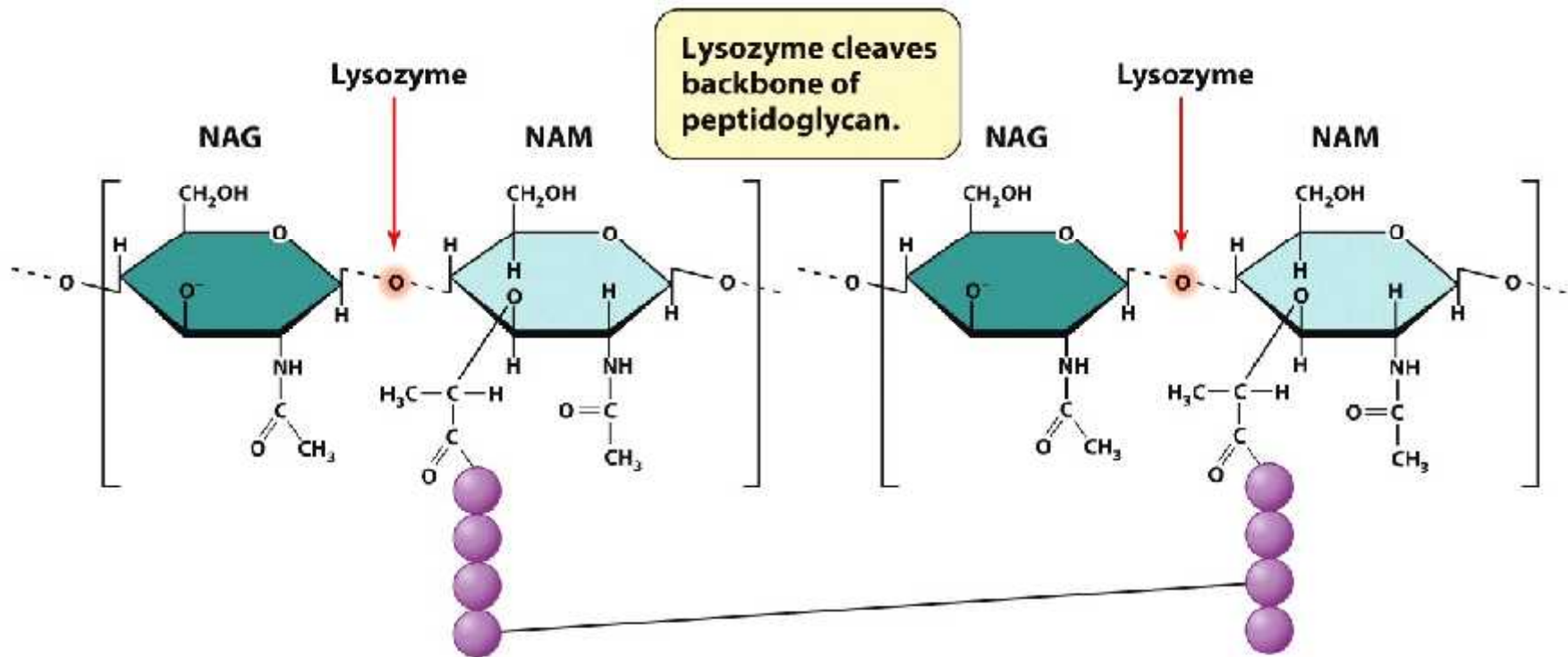 - *N*-acetylglucosamine (NAG)

The cell wall:

– How does the CW actually form?
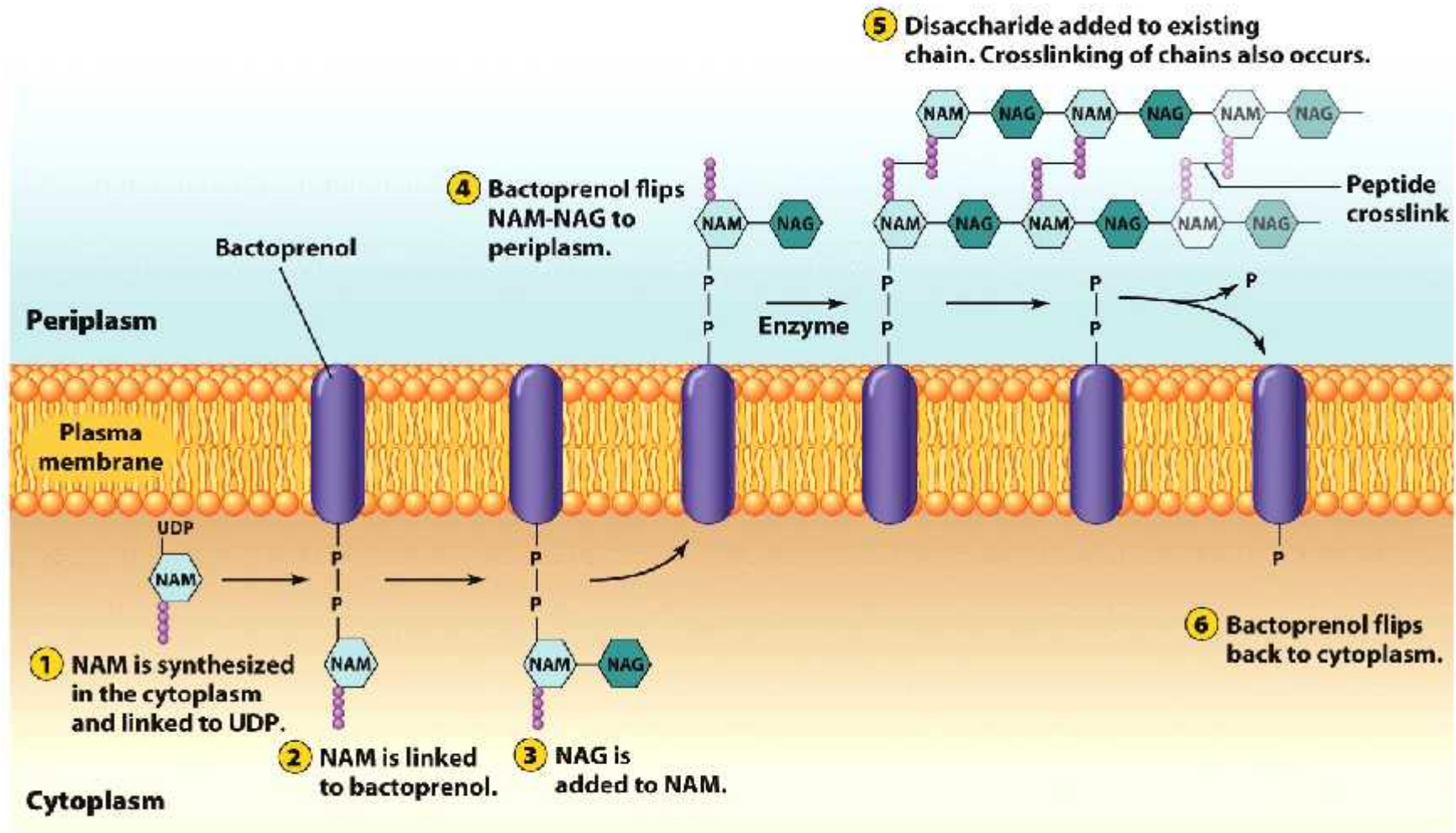
32

The cell wall :

- Can the CW structure be degraded? YES!
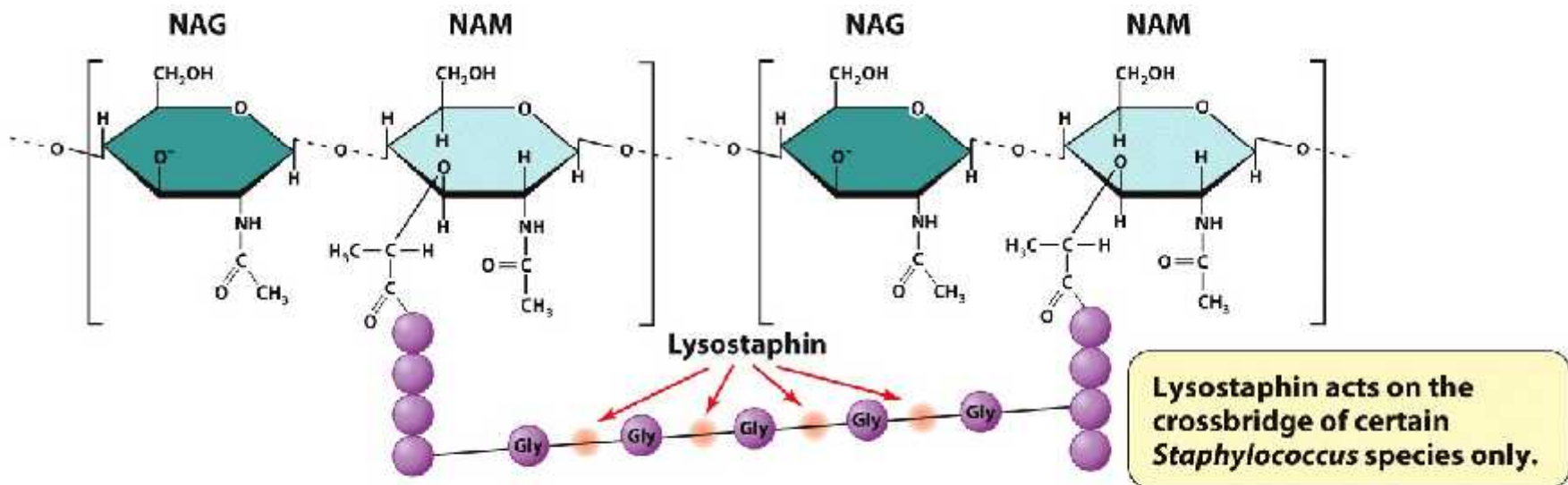 - Naturally by lysozyme and lysostaphin secretions

Lysozyme

NAG

Lysozyme cleaves backbone of peptidoglycan.

NAM

Lysozyme

NAG

NAM

CH$_2$OH

CH$_2$OH

CH$_2$OH

CH$_2$OH

NH

H$_3$C—C—H

NH

NH

H$_3$C—C—H

NH

Chemical action of lysozyme

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The cell wall :

- Can the CW structure be degraded? YES!
  - Naturally by lysozyme and lysostaphin secretions

Chemical action of lysostaphin

NAG, NAM, NAG, NAM

Lysostaphin

Lysostaphin acts on the crossbridge of certain *Staphylococcus* species only.

The cell wall :

- Can the CW structure be degraded? YES!
  - Artificially by b-lactam antibiotics
  - These work by preventing peptidoglycan crosslinking, weakening the cell wall structure.
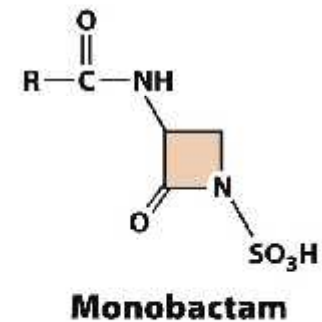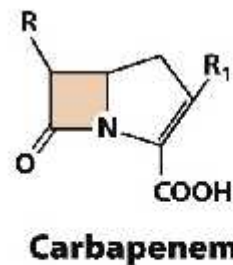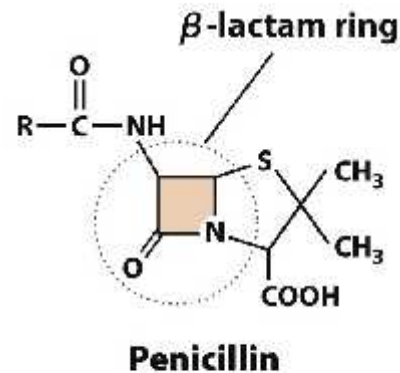
β-lactam ring

Penicillin

Cephalosporin

Carbapenem

Monobactam

β-lactam antibiotics

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The cell wall :

- So what happens when you weaken the CW?
  - The cell can't resist osmotic pressure changes.

① Lysozyme cuts peptidoglycan.

Peptidoglycan

Plasma membrane

Rod-shaped bacterium

Isotonic conditions

Protoplast

③ In hypotonic conditions, water rushes in.

Ruptured protoplast

② Without an intact cell wall, the rod shape is lost.

④ Internal pressure causes cell lysis.
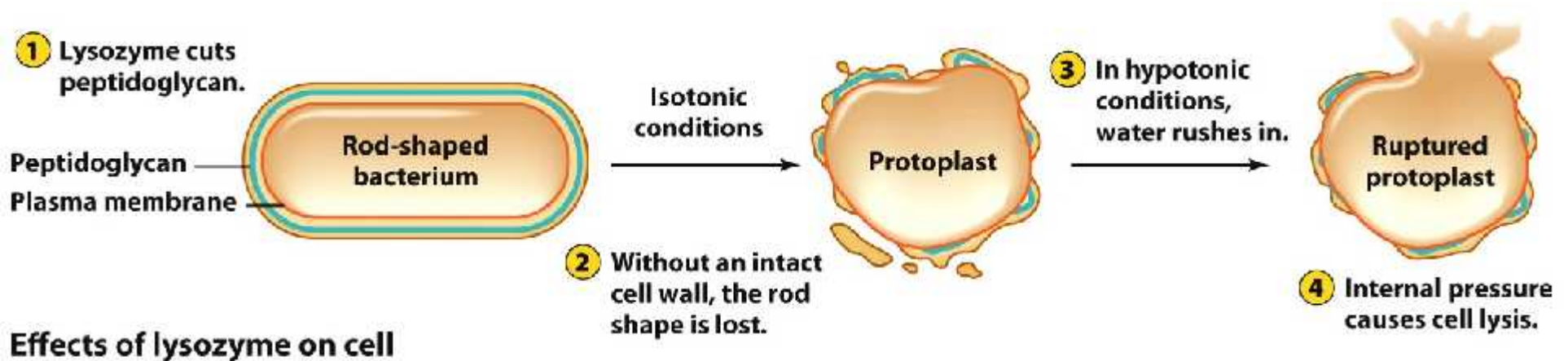
Effects of lysozyme on cell

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The cell wall :

- Wait a minute—what about antibiotic resistance?
  - Some bacteria can produce an enzyme to destroy the critical b-lactam ring structure.
  - BUT we can add a second drug to inhibit that enzyme and restore the first drug's efficiency!
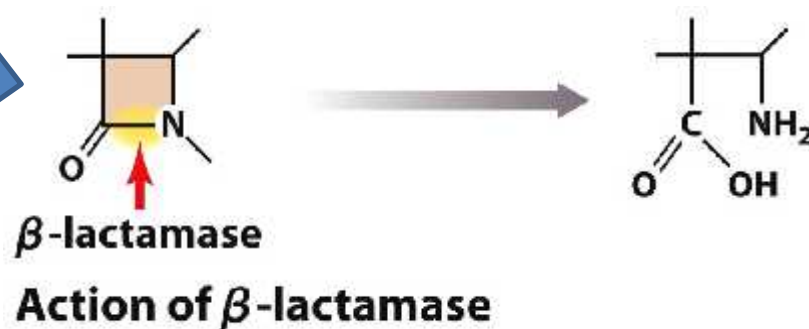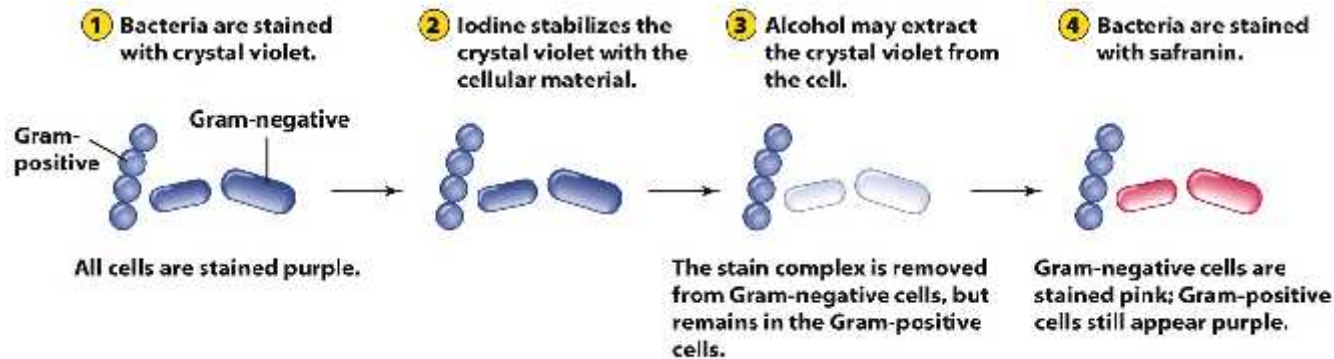
β-lactamase

Action of β-lactamase

Amoxicillin

Clavulanic acid

37
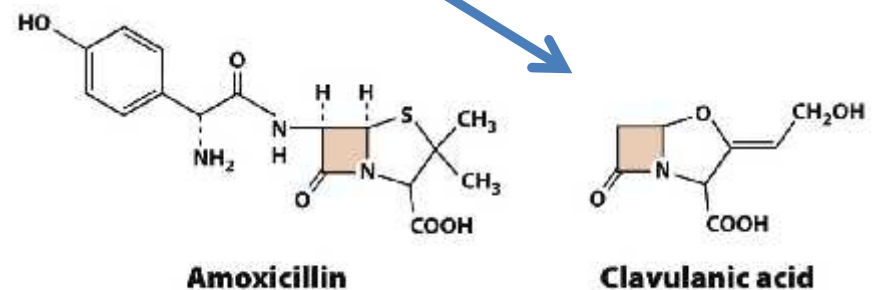
The cell wall :

- So the CW is critical, but are all CW structures the same? NO!
  - A stain method developed in 1884 by Hans Christian Gram can separate many microbes into one of two classes.

① Bacteria are stained with crystal violet.

② Iodine stabilizes the crystal violet with the cellular material.

③ Alcohol may extract the crystal violet from the cell.

④ Bacteria are stained with safranin.

Gram-positive   Gram-negative

All cells are stained purple.

The stain complex is removed from Gram-negative cells, but remains in the Gram-positive cells.

Gram-negative cells are stained pink; Gram-positive cells still appear purple.

Courtesy Christine Dupont

LM

① Bacteria are stained with crystal violet.

② Iodine stabilizes the crystal violet with the cellular material.

③ Alcohol may extract the crystal violet from the cell.

④ Bacteria are stained with safranin.

Gram-positive

Gram-negative

All cells are stained purple.

The stain complex is removed from Gram-negative cells, but remains in the Gram-positive cells.

Gram-negative cells are stained pink; Gram-positive cells still appear purple.

39

The cell wall :

- Gram-positive cells have
  - A thick outer layer of peptidoglycan
  - A very narrow periplasmic space
  - Teichoic acids in the peptidoglycan (negatively charged)
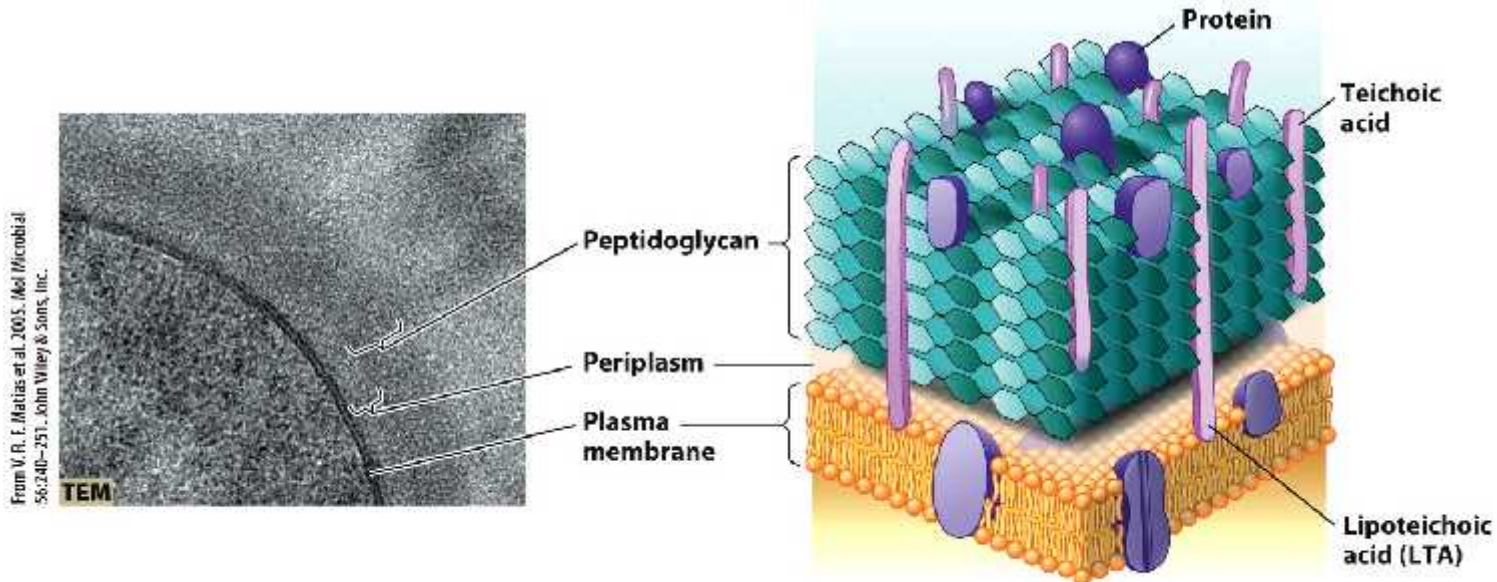
From V. R. E. Matias et al. 2005. Mol Microbial 56:240-251. John Wiley & Sons, Inc.

TEM

Peptidoglycan

Periplasm

Plasma membrane

Protein

Teichoic acid

Lipoteichoic acid (LTA)

Black, Microbiology: Principles and Explorations, copyright 2012. John Wiley & Sons, Inc.
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The cell cell:

- Gram-negative cells have
  - A varying width periplasmic space containing a very thin layer of peptidoglycan
  - An outer membrane composed of lipopolysaccharide (LPS)

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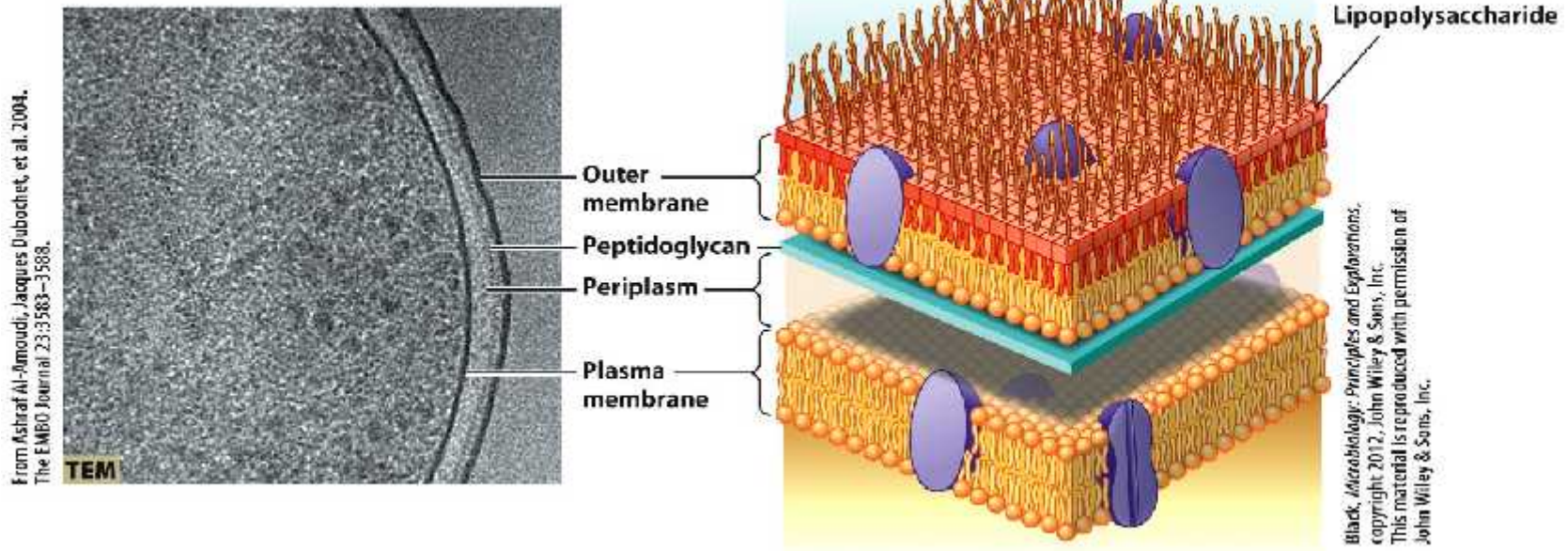
The cell cell:

- LPS from Gram-negative cells can be harmful!
  - Lipid A portion induces a strong inflammatory response.
  - O (outer) side chain of polysaccharides can vary dramatically (and even be changed by the microbe to evade host immune responses).

42
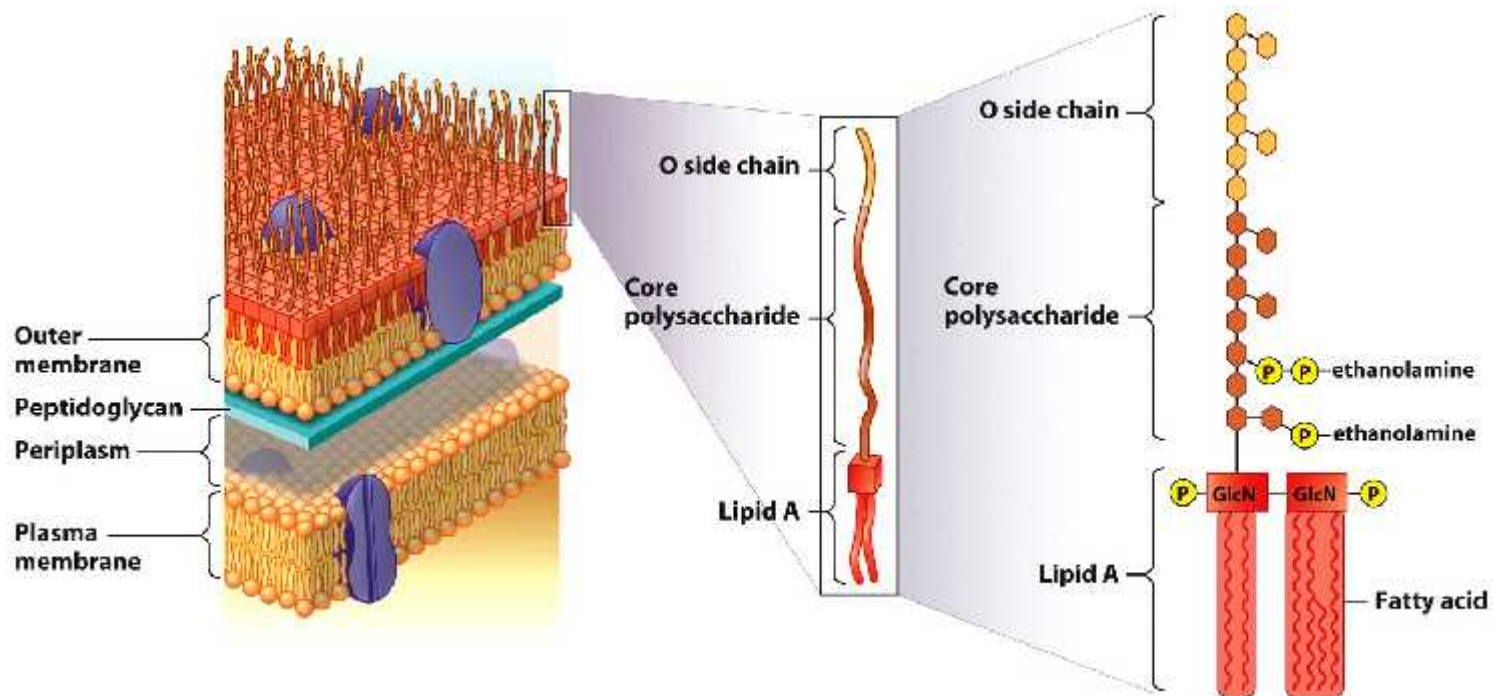
So we've covered what's IN the cell, and what separates its insides from the outside. What else is there? Lots!

TABLE 2.2 Molecules and structures on bacterial cell surfaces

Organelle or molecule	Composition	Location	Function
Lipopolysaccharide (LPS)	Lipid, polysaccharide	Outer layer of Gram-negative outer membrane; lipid portion embedded in membrane; polysaccharide exposed on surface	Stabilizes membrane; elicits an inflammatory response in the human body
Lipoteichoic acid (LTA)	Lipid, polysaccharide	Found in peptidoglycan layer of Gram-positive bacteria	Unknown; elicits an inflammatory response in the human body
Peptidoglycan	Polysaccharide backbone crosslinked with peptides	In Gram-positive bacteria, usually exposed to environment In Gram-negative bacteria, covered by the outer membrane	Maintains shape and provides structural integrity to cell
Porins	Proteins	Embedded in Gram-negative outer membrane	Form pores that allow diffusion of nutrients and water through outer membrane
TonB-dependent receptors	Proteins	Embedded in Gram-negative outer membrane	Catalyze high-affinity active transport of molecules across outer membrane
Flagella	Protein subunits	Extend outward from surface, except in spirochetes, where periplasmic flagella wrap around cell	Provide motility
Pili	Protein subunits	Extend outward from cell	Allow attachment; tip often binds to specific molecules. In some bacteria, pili are retractable and allow "twitching motility."
Capsule	Usually loose network of polysaccharides	Covers surface of cell	Protects from phagocytes; contributes to biofilm formation
Surface array (S-layer)	Protein	Covers surface of cell	May protect from bacteriophage

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The bacterial cell surface:

- *How do structures on the surface of bacterial cells allow for complex interactions with the environment?*
  - Motility from flagella: spiral, hollow, rigid filaments extending from the cell surface
    - Locations and number vary from species to species.
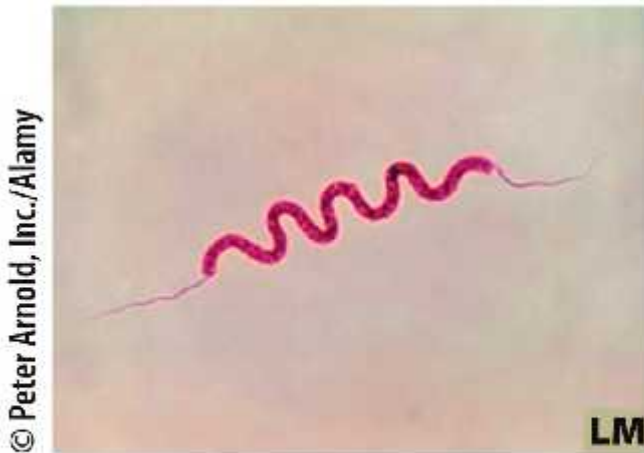
Spirillum volutans with polar flagella
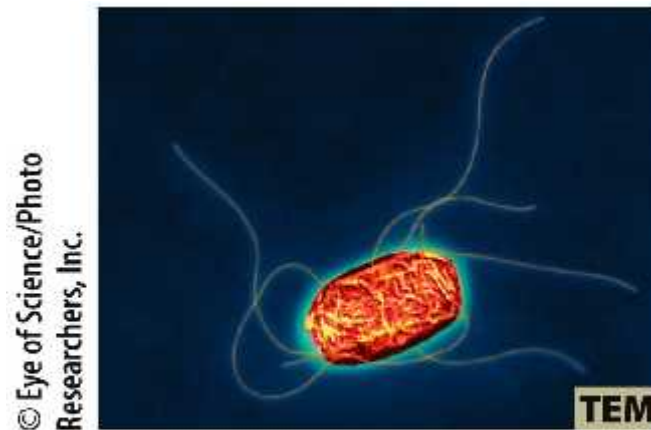
Salmonella enterica with peritrichous flagella

The bacterial cell surface:

- ## Motility from flagella
  - Composed of three basic pieces:
    - Filament of multiple flagellin proteins, 5–10 μm long
    - Hook protein portion that connects filament to basal body
    - Basal body: disk-like structure that produces torque on filament to turn it like a propeller
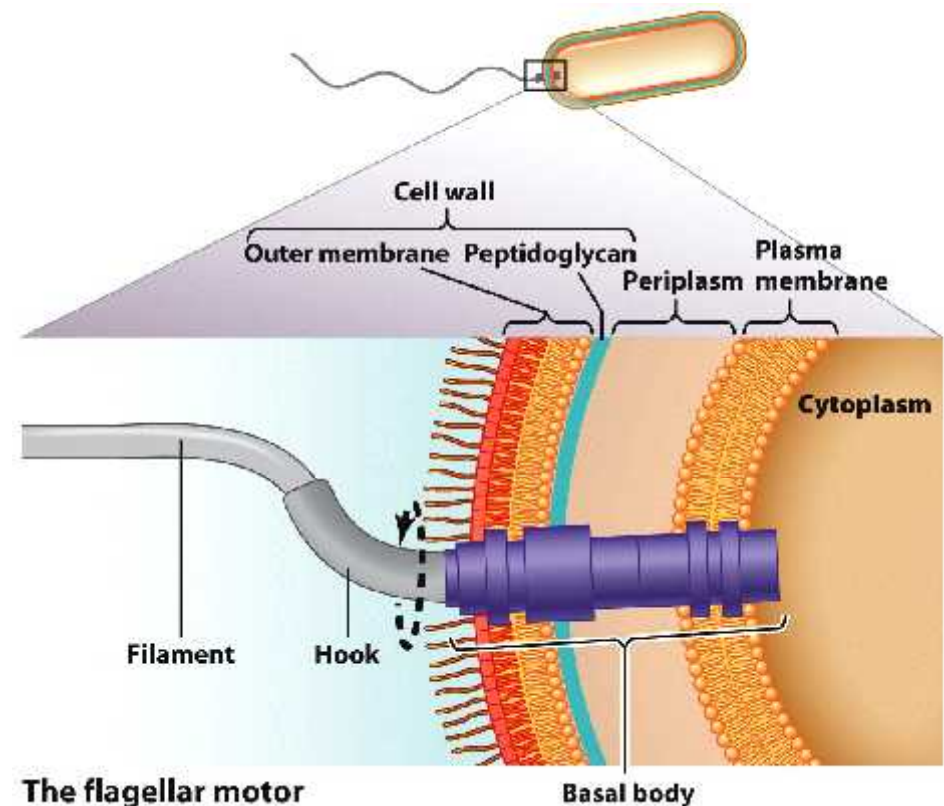
Cell wall

Outer membrane Peptidoglycan

Plasma membrane

Periplasm

Cytoplasm

Filament   Hook

The flagellar motor

Basal body

45

The bacterial cell surface:

- Motility from flagella
  - Energy to spin flagella derived from proton motive force (PMF)
  - Complex structures with up to 40 different proteins
  - Spinning one way produces a "run" (directional movement) while spinning the other way produces a "tumble" (nondirectional movement)
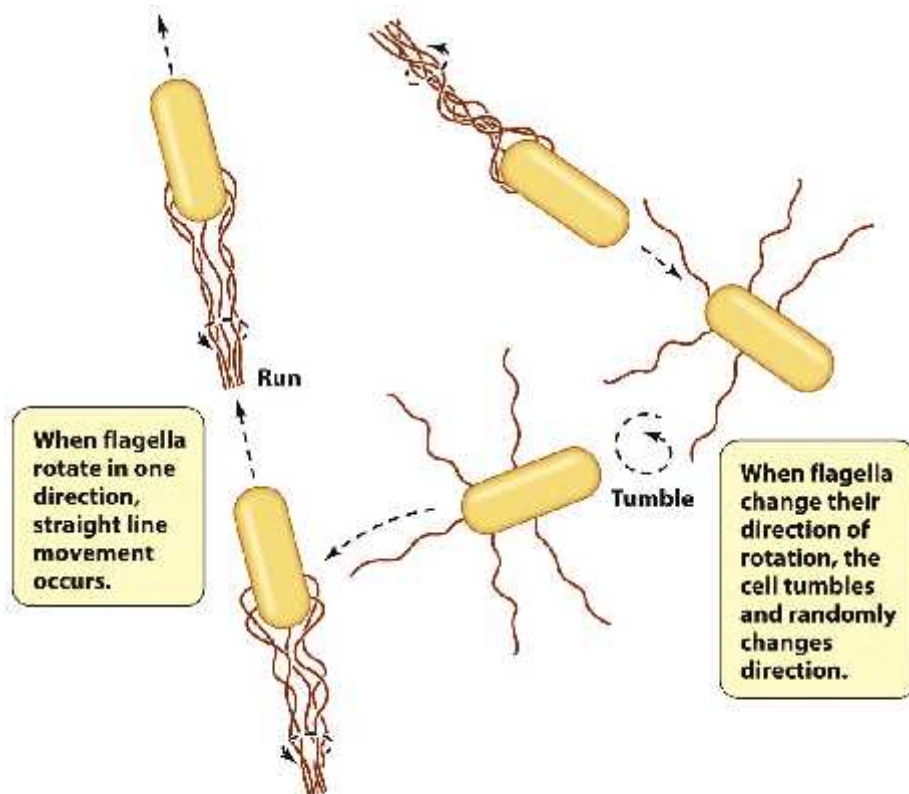
Run

When flagella rotate in one direction, straight line movement occurs.

Tumble

When flagella change their direction of rotation, the cell tumbles and randomly changes direction.

Black, *Microbiology: Principles and Explorations*, copyright 2012, John Wiley & Sons, Inc. This material is reproduced with permission of John Wiley & Sons, Inc.

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The bacterial cell surface:

- Motility from flagella
  - By using chemoreceptor proteins to sense changes in concentrations of attractants or repellents, cells can produce more runs to move in a particular direction.
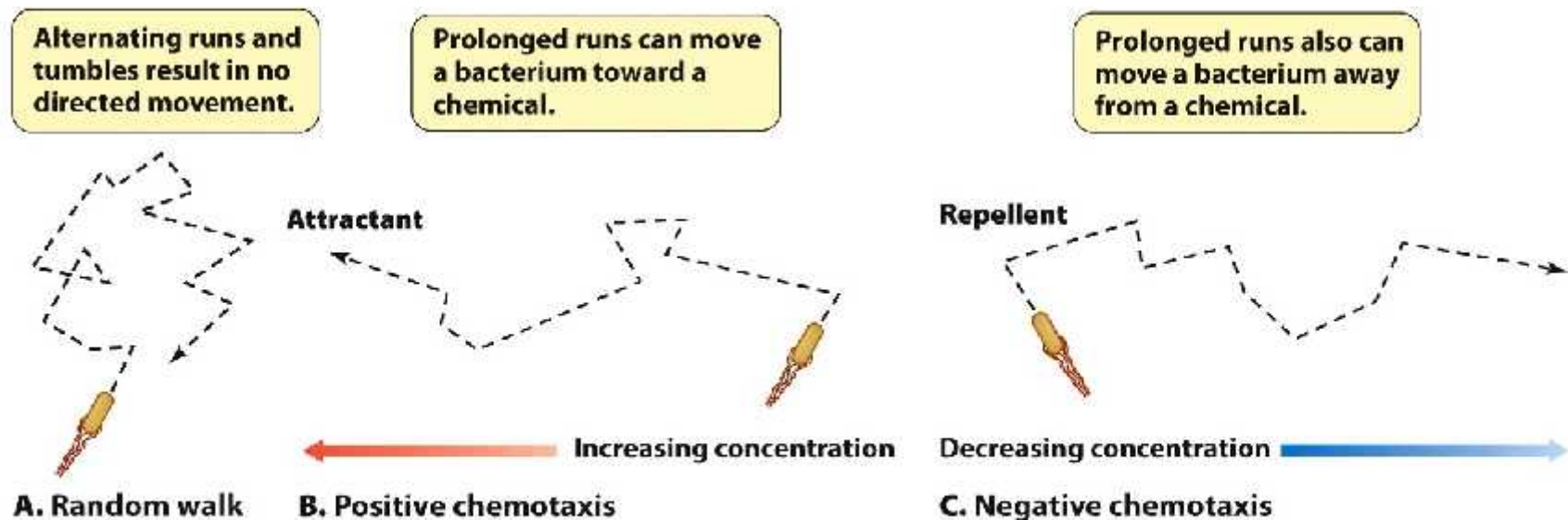
Alternating runs and tumbles result in no directed movement.

Prolonged runs can move a bacterium toward a chemical.

Prolonged runs also can move a bacterium away from a chemical.

Attractant

Repellent

Increasing concentration

Decreasing concentration

A. Random walk   B. Positive chemotaxis   C. Negative chemotaxis

The bacterial cell surface:

- Motility from flagella
  - Not all cells have EXTERNAL flagella!
  - Some spirochetes have flagella in the periplasm.
  - As they spin, they rotate the entire cell body like a corkscrew.
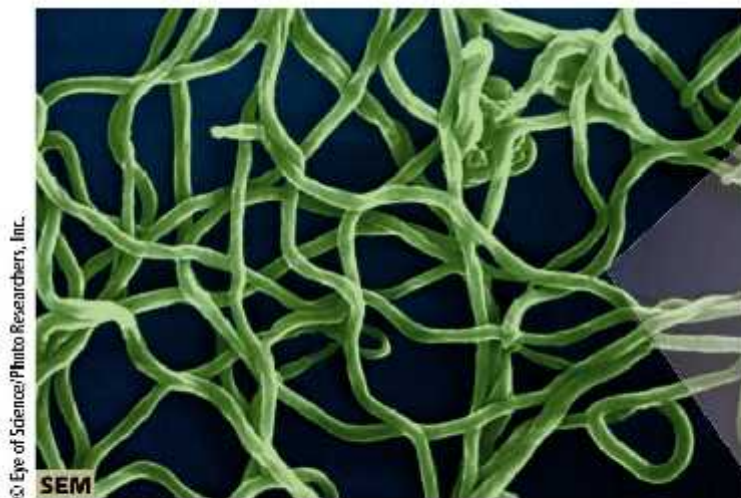
The periplasmic flagella (arrow) occupy the periplasm and extend the length of the bacterium.
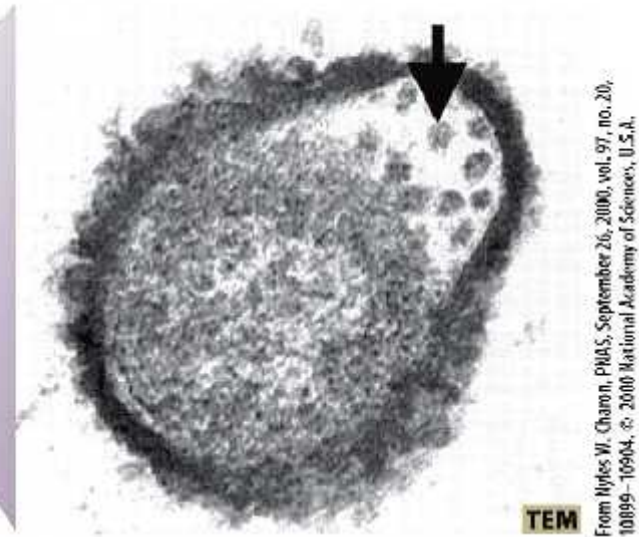
48

The bacterial cell surface:

- What about nonflagellar motility?

  – Gliding motility: smooth sliding over a surface, not well understood (*myxobacteria, cyanobacteria*)

  – Twitching motility: slow, jerky process using fibers (pili) that can be extended, attached to a surface, and pulled back to pull along a surface (*N. meningitidis, P. aeruginosa*)

The bacterial cell surface:

- What about nonflagellar motility?
  - Polymerization of actin in host cells for propulsion of bacteria into adjacent cells (*Shigella dysenteriae, Listeria monocytogenes*)
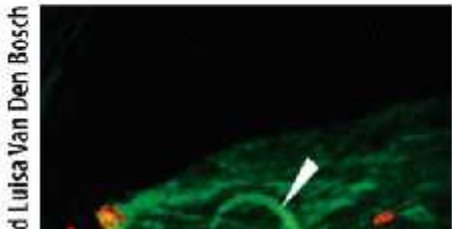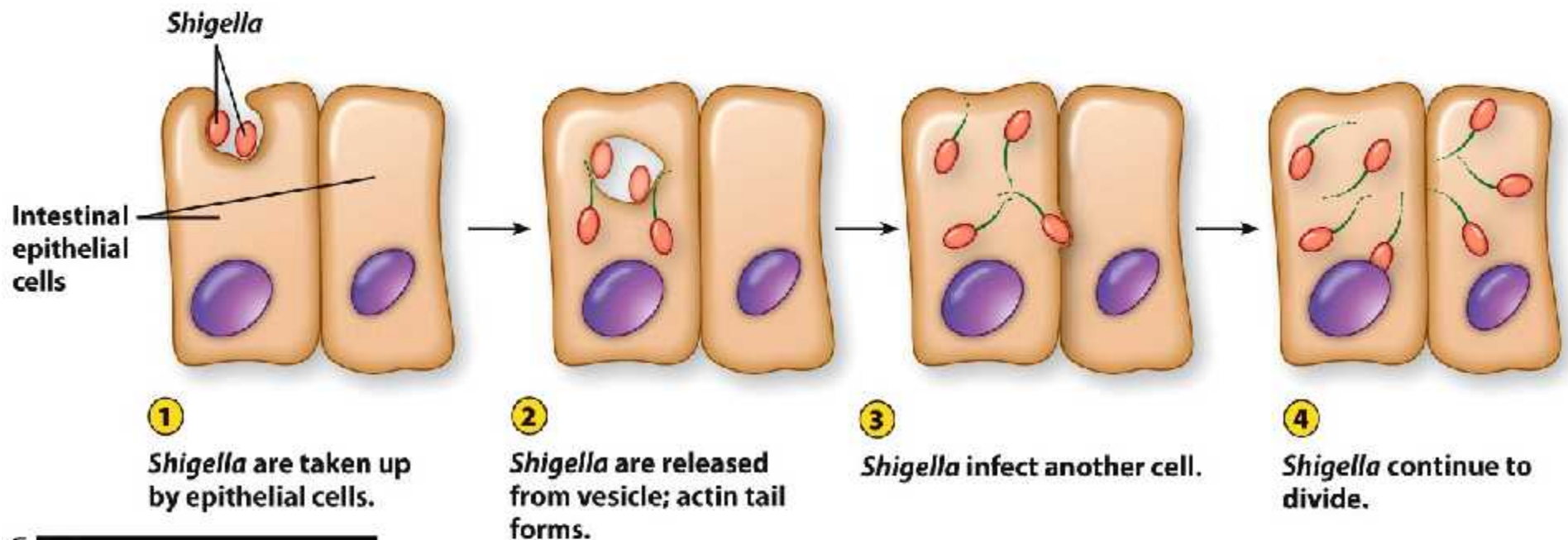
Shigella

Intestinal epithelial cells

① Shigella are taken up by epithelial cells.

② Shigella are released from vesicle; actin tail forms.

③ Shigella infect another cell.

④ Shigella continue to divide.

d Luisa Van Den Bosch

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The bacterial cell surface:

- So aside from motility, what other stuff is still left on the outside of bacterial cells?
  - Adherence molecules to stick to surfaces
    - Mediated by pili (*s.* pilus), fibers of pilin protein possess other proteins on their tips for sticking.
    - A sex pilus is a different structure used for conjugation (sending a DNA plasmid from one cell to another).
    - Some scientists prefer to use "pili" only for conjugation structures and "fimbriae" (*s.* fimbria) for adherence.
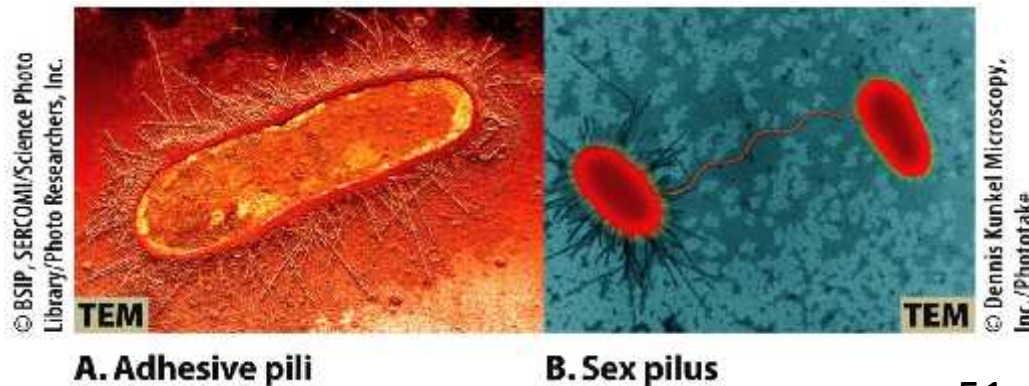
A. Adhesive pili   B. Sex pilus

© BSIP, SERCOMI/Science Photo Library./Photo Researchers, Inc.

© Dennis Kunkel Microscopy, Inc./Phototake

TEM   TEM

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The bacterial cell surface:

- So aside from motility, what other stuff is still left on the outside of bacterial cells?

  - *Capsules: thick layer of polysaccharides surrounding some cells*

    - Can provide adhesion, defense against host immunity, protection against drying out (desiccation)

A. Capsules surrounding *Streptococcus pneumoniae*

B. Capsular material surrounding *Streptococcus zooepidemicus*
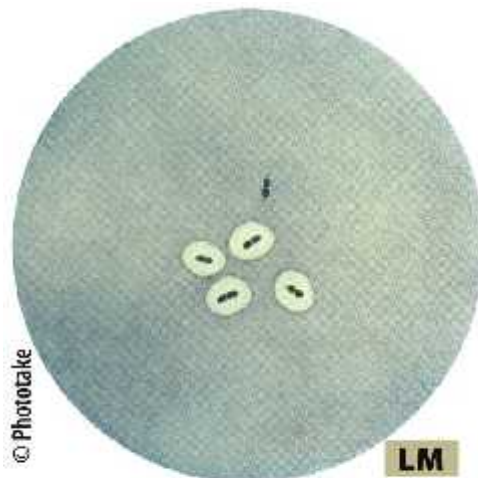
52

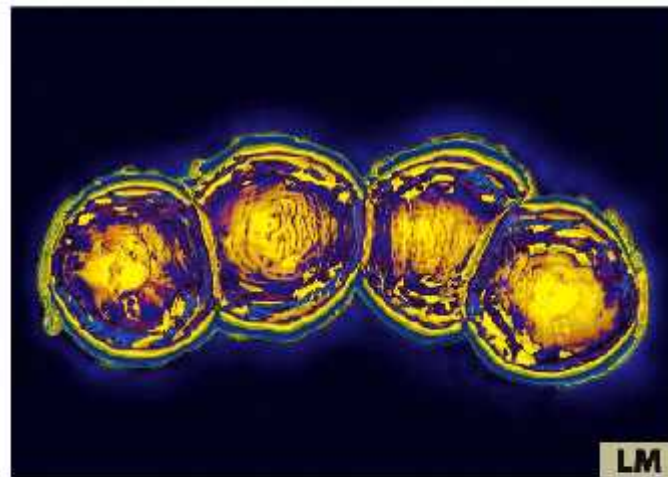# The bacterial cell surface:

- So aside from motility, what other stuff is still left on the outside of bacterial cells?
  - Capsules can help bacteria form biofilms.
    - Biofilms provide protection and enhanced survivability in harsh environments.
    - Examples of biofilms include dental plaque and mold on bathroom surfaces.
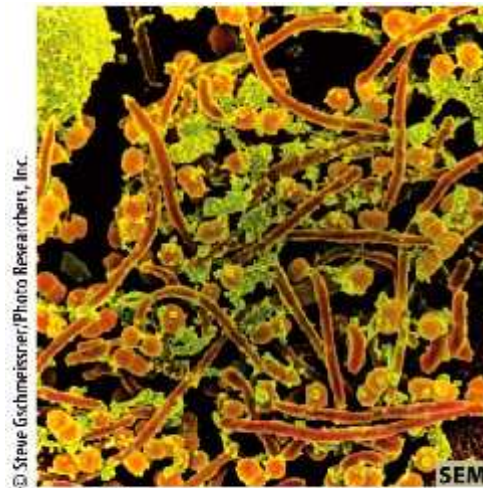
A. Biofilm on human tooth
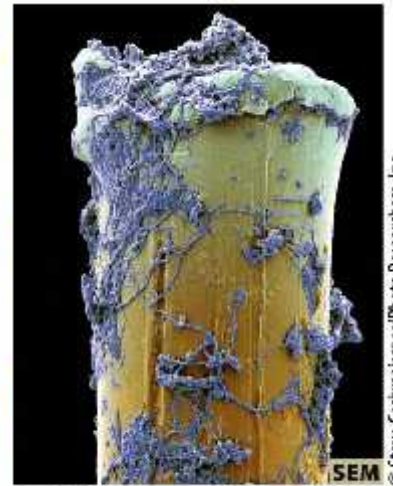
B. Biofilm on a toothbrush bristle

Endospores

- Some bacteria, notably *Bacillus* and *Clostridium* are characterized by the ability to produce unique structures called endospores, which are important for several reasons, including their durability and potential pathogenicity.

- A single bacterial cell, called a *vegetative cell to distinguish* it from an endospore, transforms into only one endospore, which then germinates to grow into only one vegetative cell; therefore, endospores are not reproductive structures. Instead, endospores constitute a defensive strategy against hostile or unfavorable conditions

This section was taken from **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**

Endospores

- Endospores are extremely resistant to drying, heat, radiation, and lethal chemicals. For example, they remain alive in boiling water for several hours; are unharmed by alcohol, peroxide, bleach, and other toxic chemicals; and can tolerate over 400 rad of radiation, which is more than five times the dose that is lethal to most humans.

This section was taken from **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**
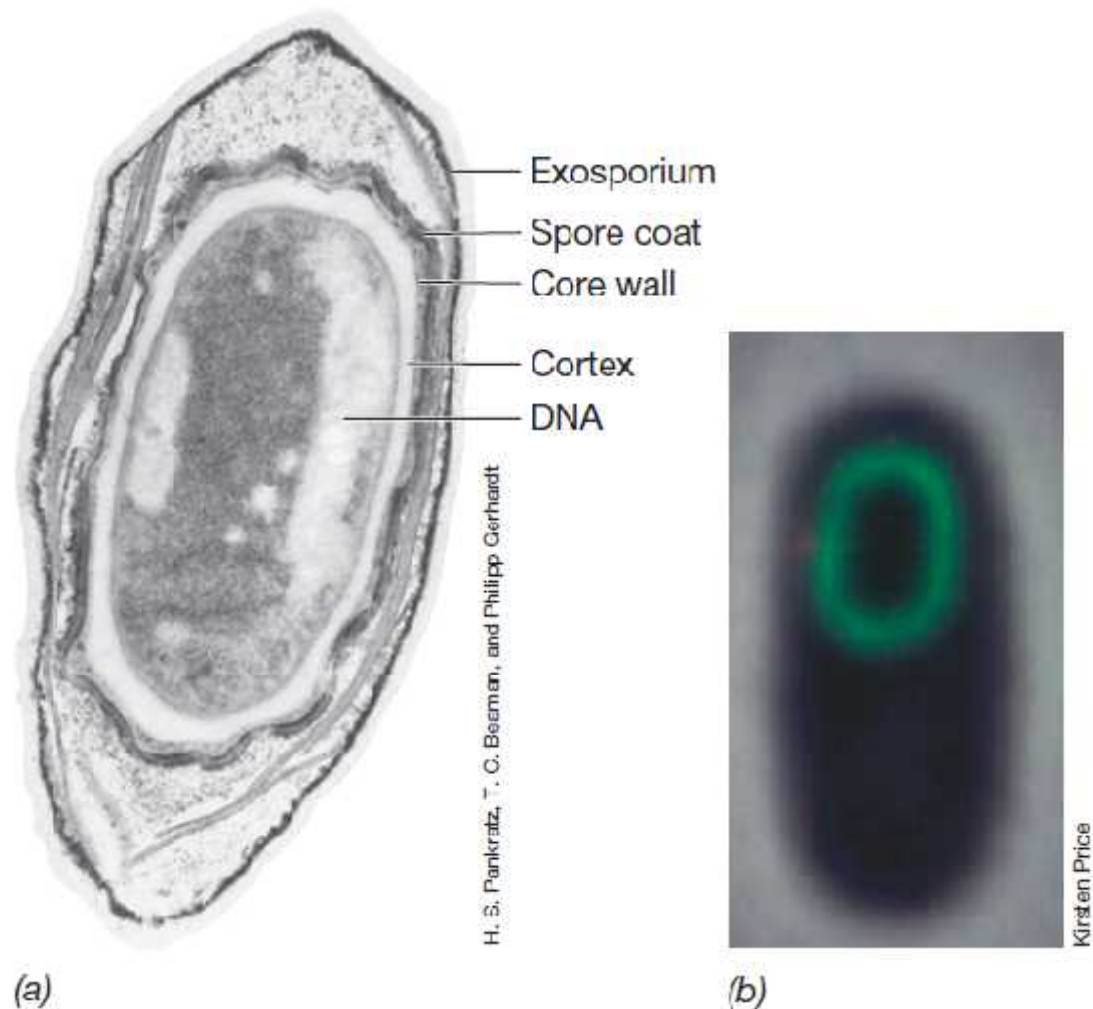
Figure 3.35 Structure of the bacterial endospore. (a) Transmission electron micrograph of a thin section through an endospore of *Bacillus megaterium*. (b) Fluorescent photomicrograph of a cell of *Bacillus subtilis* undergoing sporulation. The green color is a dye that specifically stains a sporulation protein in the spore coat.

Bacterial taxonomy:

- *How are bacteria categorized and named?*
  - Important! Remember that most microbes still can't be cultured!
  - What we CAN grow, we name according to the standard binomial system.
    - Species: group of strains sharing common features, while differing considerably from other strains
    - Genus: group of closely related species

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Bacterial taxonomy:

- *How are bacteria categorized and named?*
  - Above the genus level, we use family, order, class, phylum, and finally domain.

TABLE 2.3 **Classification hierarchy within the Domain Bacteria, using *Brucella melitensis* as an example**

Taxonomic level	Example
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rhizobiales
Family	Brucellaceae
Genus	*Brucella*
Species	*melitensis*

Bacterial taxonomy:

- *How are bacteria categorized and named?*
  - Classification depends on many features:
    - Size/shape
    - Gram type
    - Colony morphology
    - Presence of structures such as capsules/endospores
    - Physiologic/metabolic traits (see Ch. 13)
    - DNA sequence data (in more recent years)

Bacterial taxonomy:

- Once classified, microbes are deposited in at least two culture collections.
  - The World Federation for Culture Collections maintains a database of more than 500 collections from over 60 countries.
  - These are pure, maintained cultures made available to scientists for research purposes.

Microbes in the environment:

- Ecosystems
  - Interactions and exchanges of materials between organisms and their surrounding environment
    - Community of organisms
      - Primary producers capture energy through photosynthesis.
      - Consumers ingest/utilize stored photosynthetic energy.
      - Decomposers recycle components back into environments.
    - Members of communities can be grouped into functional groups called "guilds."
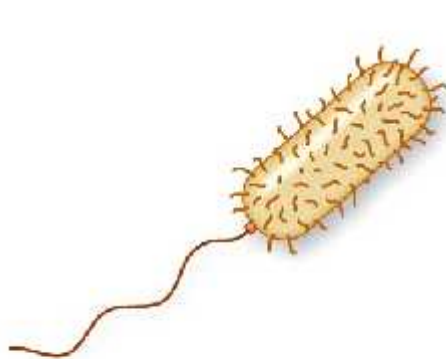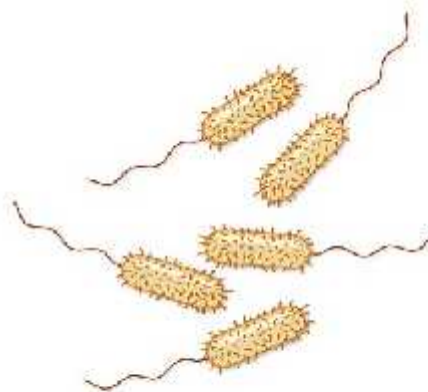
Individual   Population   Community
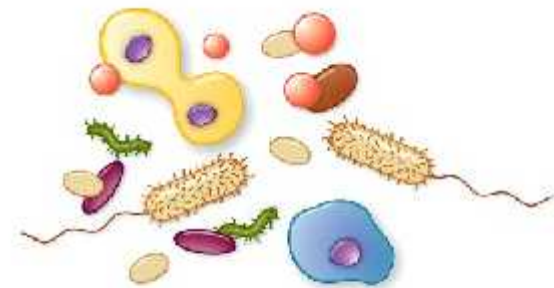
- Biofilms
  - Groups/layers of microbes on a surface that interact with and support each other
  - Can be found in nature often but can be of practical importance to humans who want to rid a surface of microbes

Courtesy Christine Dupont

Biofilm

Figure 4.23b Microbiology: An Evolving Science
©J. D. Ruby, K. F. Gerencser

① Adhesion by a primary colonizer occurs.

② Cells divide.

③ Microcolony produces exopolysaccharide (EPS).

Exopolysaccharide

④ Introduction of secondary colonizers and growth of the biofilm community.

Solid surface
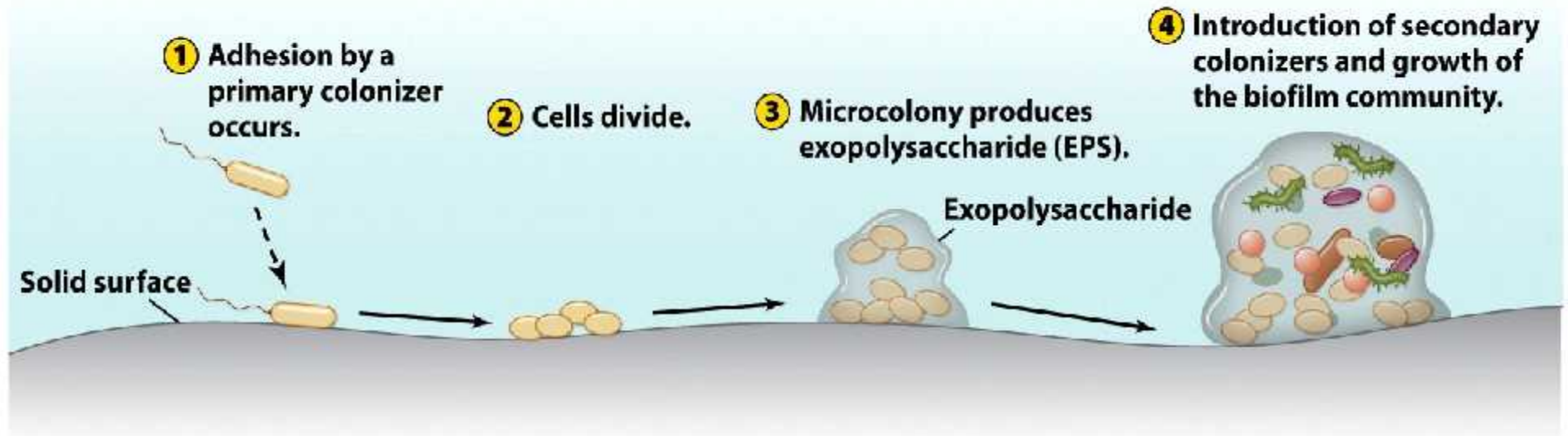
- **Biofilms**
  - Formation often begins with appendaged bacteria forming the primary layer on a surface.
  - Secondary colonizers then join the biofilm.
  - Together microbes secrete exopolysaccharide (EPS).
    - EPS helps protect the biofilm but also helps form water-filled channels for transport of nutrients and wastes.

- Biofilms: EPS colanic acid and its importance in biofilm formation in *E. coli* K12

Observation: *E. coli* K12 forms biofilms and produces the exopolysaccharide colanic acid.

SEM

E. coli biofilm

© SciMAT/Photo Researchers, Inc.

Hypothesis: Colanic acid plays a vital role in biofilm formation.

Experiment: Examine the biofilm formation of wild-type *E. coli* K12 and a mutant that does not produce colanic acid.

Wild-type *E. coli*

Mutant *E. coli*

Colanic acid
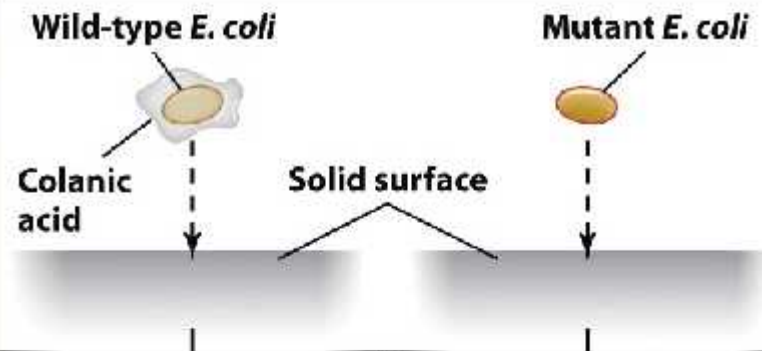
Solid surface

Results: The wild type was able to form a complex biofilm. Although the mutant attached to the solid surface, it did not form a complex biofilm.

Conclusion: In *E. coli* K12, colanic acid is necessary for biofilm formation.

Complex biofilm formation

Attachment, but no biofilm formation
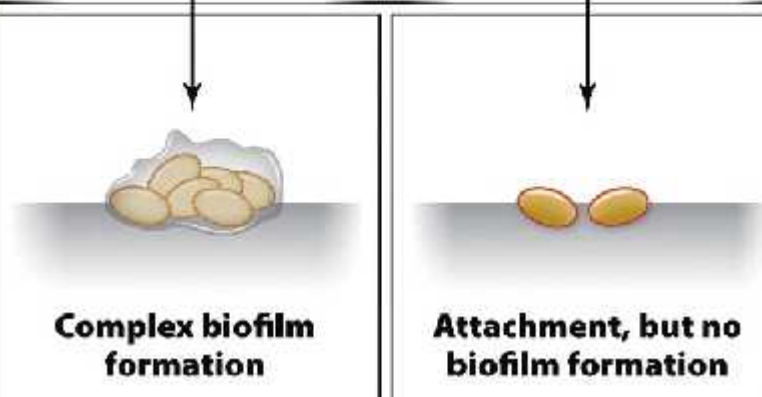
One of the most clinically and industrially relevant properties of biofilm microbial communities is their **inherent tolerance to antibiotics** and other antimicrobial stressors.

A given species growing in a biofilm can be up to 1000 times more tolerant of an antimicrobial substance than planktonic cells of the same species.

Besides cystic fibrosis, biofilms have been implicated in several medical and dental conditions, including kidney stones, tuberculosis, and *Staphylococcus* infections**.**

- Medical implants are ideal surfaces for biofilm development. These include both short-term devices, such as a urinary catheter, as well as long-term implants, such as artificial joints. It is estimated that 10 million people a year in the United States experience biofilm infections from implants or intrusive medical procedures

- Biofilms explain why routine oral hygiene is so important for maintaining dental health. Dental plaque is a typical biofilm and contains acid-producing bacteria responsible for dental caries

Microbiology for Nursing students

Chapter Two: Microorganisms Growth and Cultivating
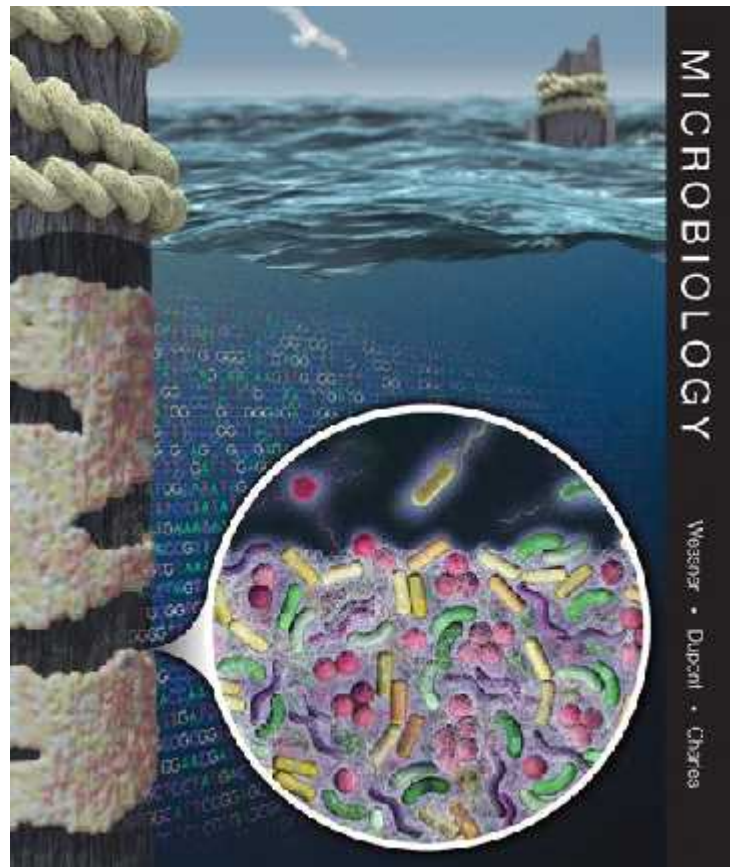
Dr. Sulaiman Alnaimat 2015

Nutritional requirements of microorganisms:

- *What do microorganisms generally need to grow?*
  - All cells need access to large amounts of carbon, nitrogen, phosphorus, sulfur, and oxygen (macronutrients) to build macromolecules.
  - Various micronutrients are also required by microbes:
    - Includes several metal ions (Na^+, Mg^{2+}, Mn^{2+}, etc.)
    - Often required for protein structure/activity

- Acquisition of nutrients
  - Autotrophs assimilate carbon from inorganic sources.
  - Heterotrophs assimilate carbon in preexisting organic form.

3
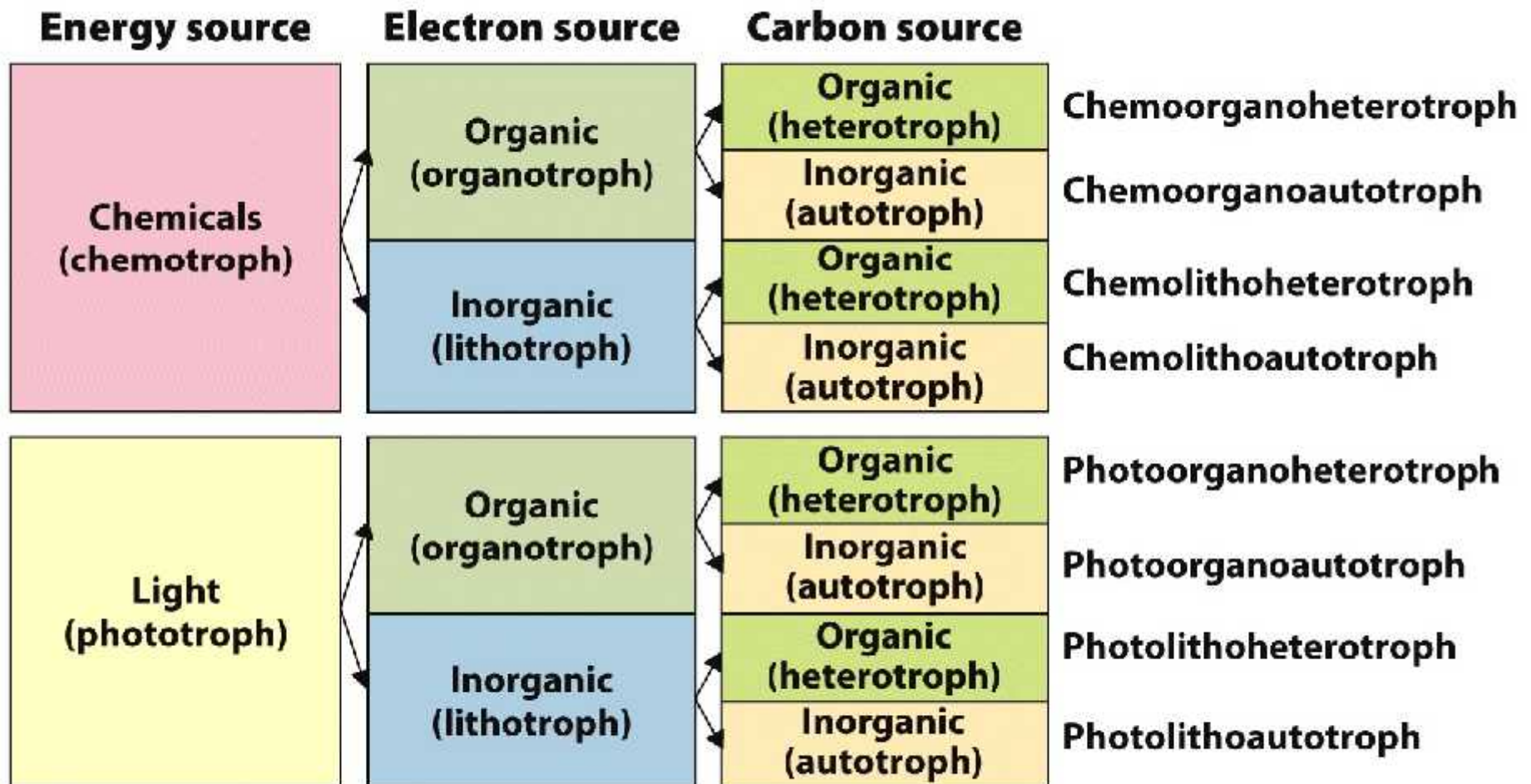
Factors affecting microbial growth:

- Nutrient concentration
  - Growth rate will depend on the amounts of nutrients in the environment.
  - One key nutrient, available in the lowest amount, will dictate how much growth can occur over time (i.e., it will be a limiting factor).
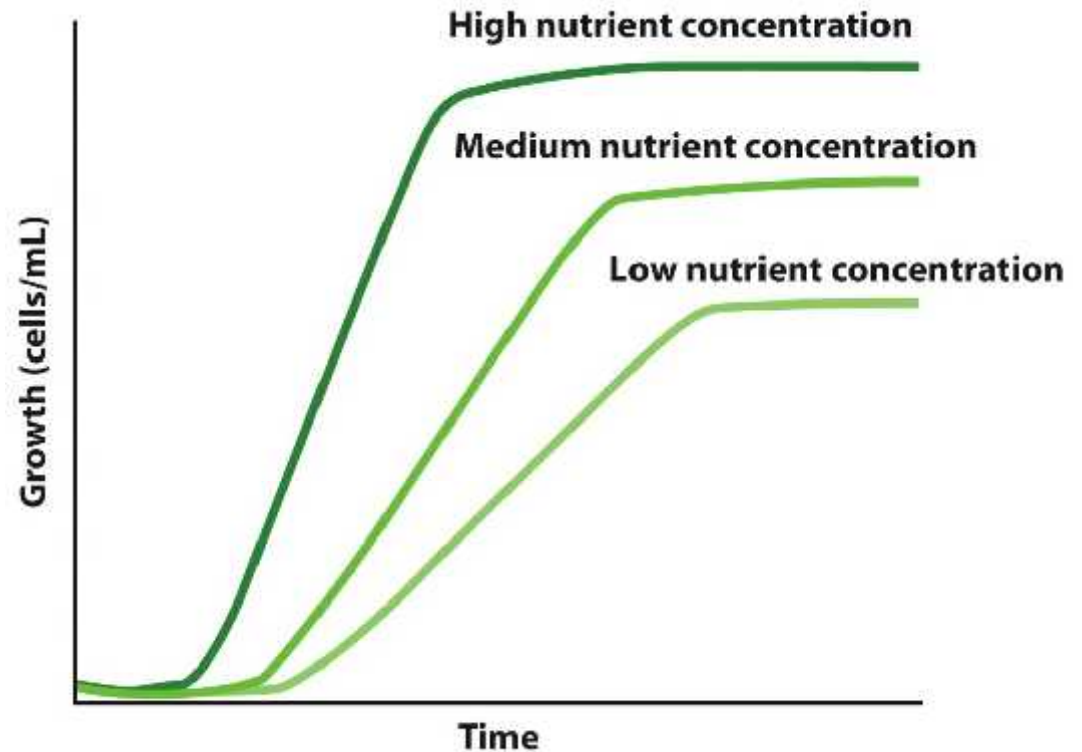
High nutrient concentration

Medium nutrient concentration

Low nutrient concentration

Growth (cells/mL)

Time

4

Factors affecting microbial growth:

- Effects of oxygen on microbial growth
  - Aerobes grow in the presence of oxygen.
    - Obligate aerobes REQUIRE oxygen.
    - Microaerophiles grow best when there is less oxygen than normal.
  - Anaerobic growth occurs without oxygen.
    - Aerotolerant anaerobes aren't harmed by oxygen but don't use it, either.
    - Obligate anaerobes cannot grow when oxygen is present.
    - Facultative anaerobes CAN use oxygen but can also grow in the absence of oxygen.

This Figure was taken from **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**

Clamp

Airtight lid

$2 H_2 + O_2 \longrightarrow 2 H_2O$

Chamber

Palladium pellets to catalyze reaction removing O_2

CO_2

O_2

H_2

Envelope containing chemicals to release CO_2 and H_2

Methylene blue (anaerobic indicator)

Petri plates
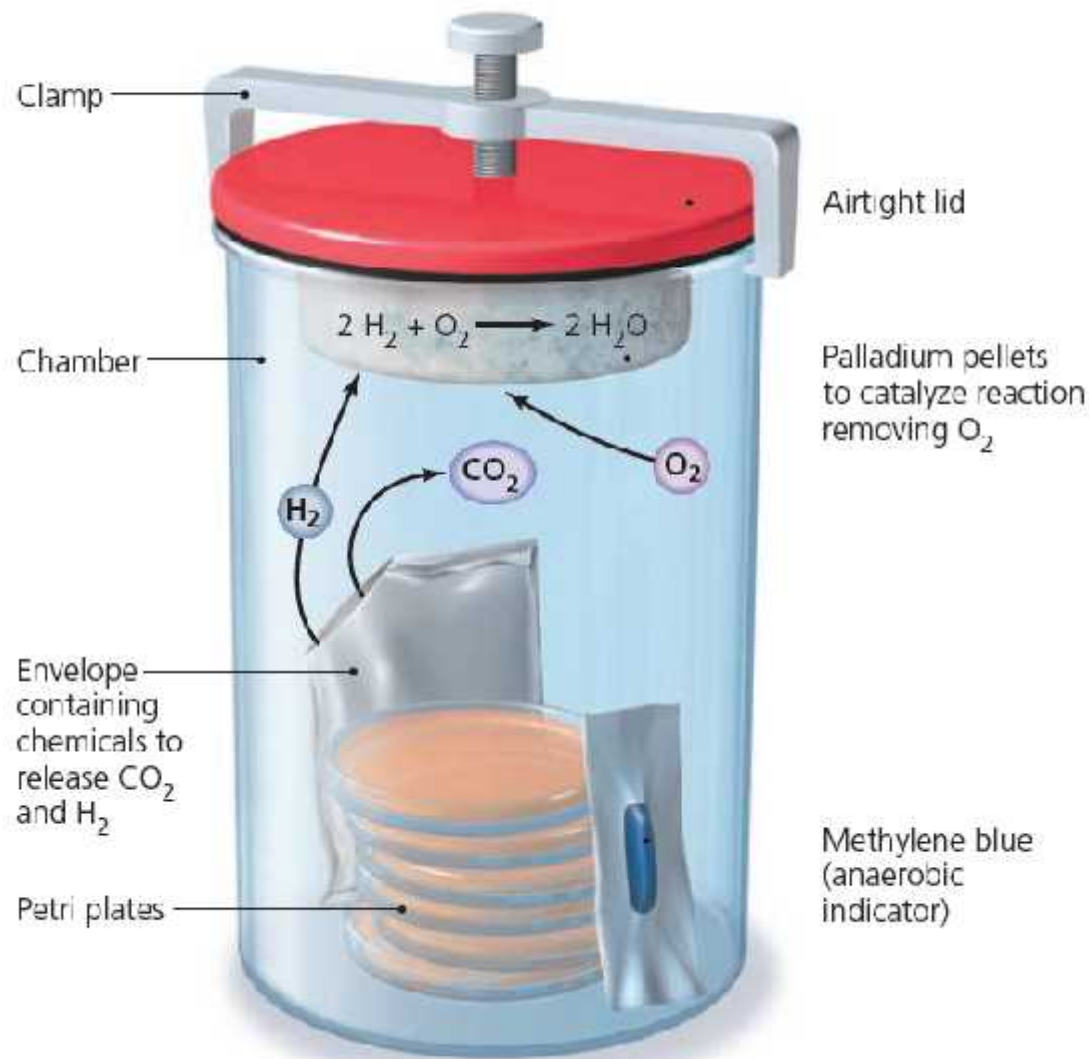
▲ **FIGURE 6.16** An anaerobic culture system. The system utilizes chemicals to create an anaerobic environment inside a sealable, airtight jar. Methylene blue, which turns colorless in the absence of oxygen, indicates when the environment within the jar is anaerobic.

6

Factors affecting microbial growth:

- Effects of oxygen on microbial growth
  - Often determined by what defenses are available against oxygen's negative effects in the cell

TABLE 6.1 Toxic oxygen species

Toxic species	Sources	Cellular defenses
Singlet oxygen: $^1O_2\cdot$	Photochemical reaction; product of peroxidase enzymes	Antioxidants such as carotenoid pigments
Superoxide anion: O_2^-		Superoxide dismutase, superoxide reductase enzymes
Hydroxyl radical: $OH\cdot$	By-products of reduction of O_2 during respiration and other biochemical redox reactions	Antioxidants such as glutathione
Hydrogen peroxide: H_2O_2		Catalase and peroxidase enzymes

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Factors affecting microbial growth:

- Effects of pH on microbial growth
  - pH affects macromolecule structures and transmembrane electrochemical gradients.
  - Each microbe will have an optimal pH range for growth.
    - Acidophiles = pH < 5.5
    - Neutrophiles = pH 5.5 to 8.5
    - Alkalophiles = pH > 8.5
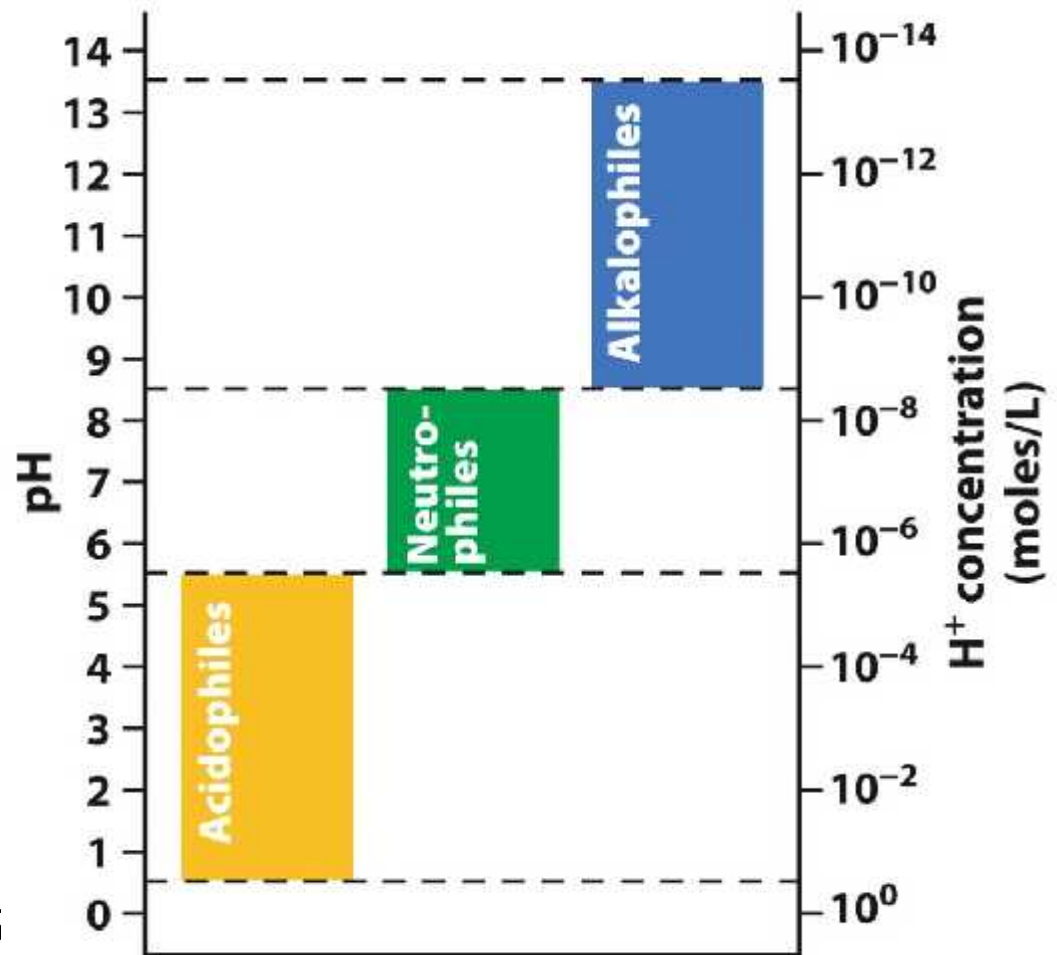
Factors affecting microbial growth:

- Effects of osmotic pressure and water availability on microbial growth
  - Different solute concentrations can result in influx of water into or efflux from the cell.
    - This can cause stress to the cell, causing it to either swell or shrink.
  - Water must also be available for biochemical reactions (measured in terms of water availability or a_w).
    - Interactions with solutes can decrease aw values.
    - Pure water a_w = 1.0; seawater a_w = 0.98; honey a_w = 0.6
    - Most bacteria require an a_w > 0.9

9

Factors affecting microbial growth:

- Effects of temperature on microbial growth
  - Temperature can also affect macromolecular structure, membrane fluidity, and enzyme function.
  - Different microbes have different optimal temperature growth ranges.

10
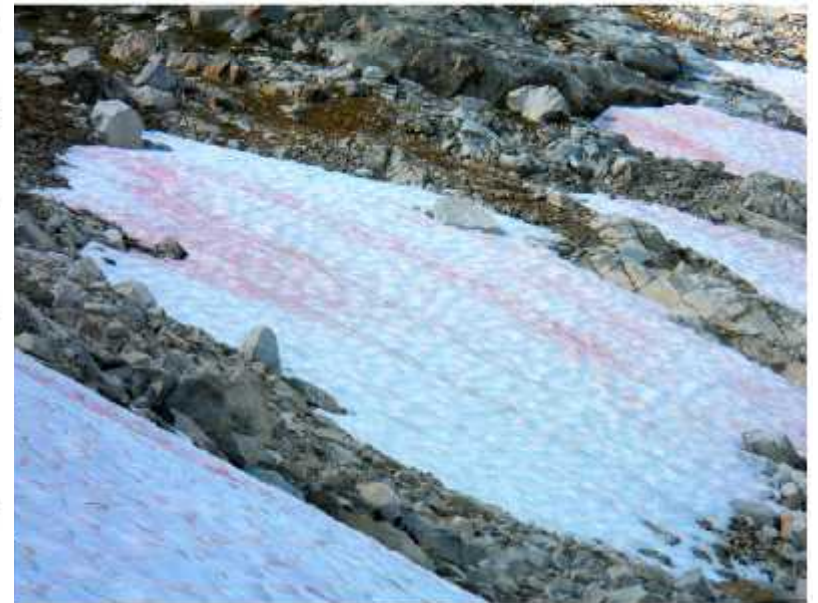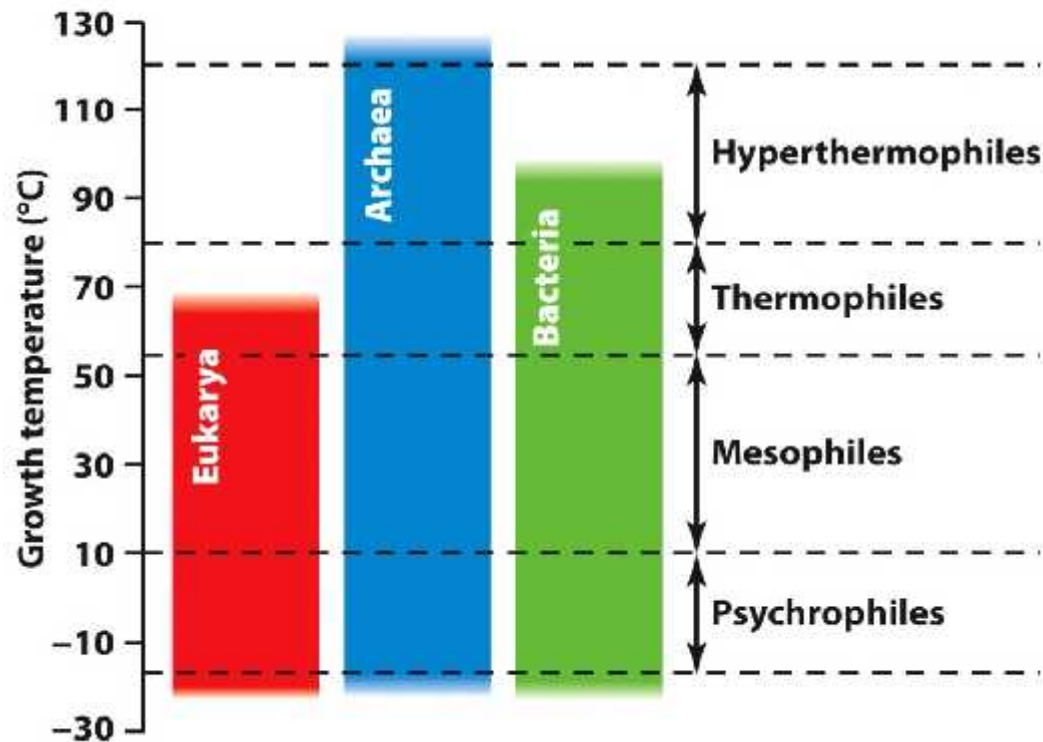
Growing microorganisms in the laboratory:

- *How can different types of microorganisms be grown in the laboratory?*
  - Media for microbial growth
  - Microbes can be grown in the lab on both solid (agar plates) and liquid media (broths).

1 Powdered agar is added to a flask containing liquid media to a final concentration of 1.5 g/100 mL (1.5%).

2 The medium is heated to above 85°C to melt the agar.

3 The medium is allowed to cool to around 45°C, then poured into a plate and allowed to solidify.

Courtesy Christine Dupont

Refined, dehydrated agar

Growing microorganisms in the laboratory:

- Media for microbial growth
  - Microbes can be grown in the lab on both solid (agar plates) and liquid media (broths).

Compact circular colonies

Filamentous colonies
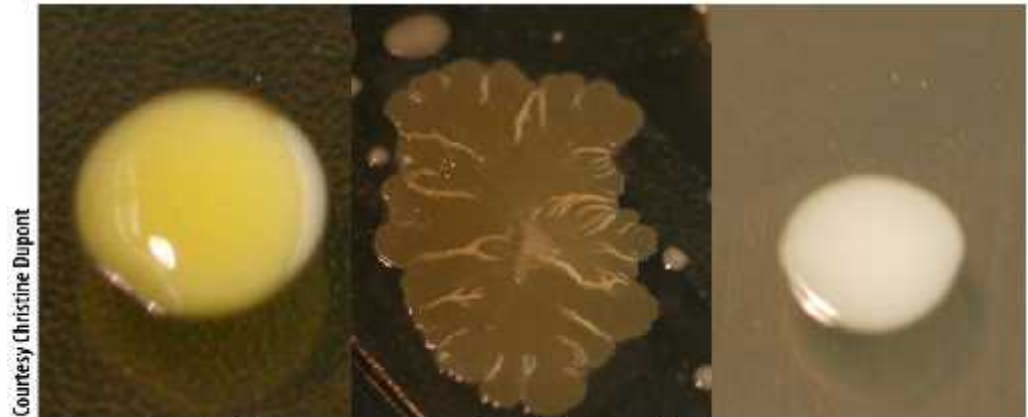
Variations in form, texture, elevation, and color

- Media for microbial growth
  - Can be either complex (unknown chemical composition) or defined/synthetic (precisely defined chemical composition)

TABLE 6.2 Complex and defined media for growing *Escherichia coli*

LB broth (per liter)		M9 minimal salts broth (per liter)	
Peptone	10 g	Glucose ($C_6H_{12}O_6$)	2 g (11 mM)
Yeast extract	10 g	Na_2HPO_4	6 g (42 mM)
NaCl	5 g	KH_2PO_4	3 g (22 mM)
(pH adjusted to 7.0)		NaCl	0.5 g (9 mM)
		NH_4Cl	5 g (93 mM)
		$MgSO_4$	1 mM
		$CaCl_2$	0.1 mM
		(pH adjusted to 7.0)	

Growing microorganisms in the laboratory:

- Specialized media
  - Selective media allows for isolation of microbes with specific properties (e.g., salt tolerance of on mannitol salt agar plates).
  - Differential media allows certain microbes to be recognized based on visual reactions in the medium (e.g., lactose fermentation of *E. coli* on MacConkey agar).
  - Enriched media can be used to increase a particular population of microbes with a specific property from a mixture of cell types (Sabouraud dextrose agar for fungi).

TABLE 6.3 Common specialized media

Medium	Organism(s) identified	Selectivity and/or differentiation achieved
Brilliant green agar © Fancy/Alamy Limited	*Salmonella*	**Selective** Brilliant green dye inhibits Gram-positive bacteria and thus selects Gram-negative ones. **Differential** Differentiates *Shigella* colonies (which do not ferment lactose or sucrose and are red to white) from other organisms that do ferment one of those sugars and are yellow to green.
Eosin methylene blue agar (EMB) Carolina Biological Supply Company/ Phototake	**Gram-negative enterics** (*Enterobacteriaceae*)	**Selective** Medium partially inhibits Gram-positive bacteria. **Differential** Eosin and methylene blue differentiate among organisms: *Escherichia coli* colonies are purple and typically have a metallic green sheen; *Enterobacter aerogenes* colonies are pink, indicating that they ferment lactose; and colonies of other organisms are colorless, indicating they do not ferment lactose.
MacConkey agar © Science PR/Getty Images, Inc.	**Gram-negative enterics**	**Selective** Crystal violet and bile salts inhibit Gram-positive bacteria. **Differential** Lactose and the pH indicator neutral red (red when acidic) identify lactose fermenters as red colonies and non-fermenters as white or tan. Most intestinal pathogens are non-fermenters and hence do not produce acid.

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Growing microorganisms in the laboratory:

- Obtaining a pure culture
  - One of the benefits of a solid medium is that cells are held in place on the surface and can be isolated.
  - This can lead to separating a mixture of cells into a pure population.
  - There are three basic methods for separating cells on a plate.
    - Streak plate method
    - Spread plate method
    - Pour plate method

Growing microorganisms in the laboratory:

- Obtaining a pure culture: Streak plate method
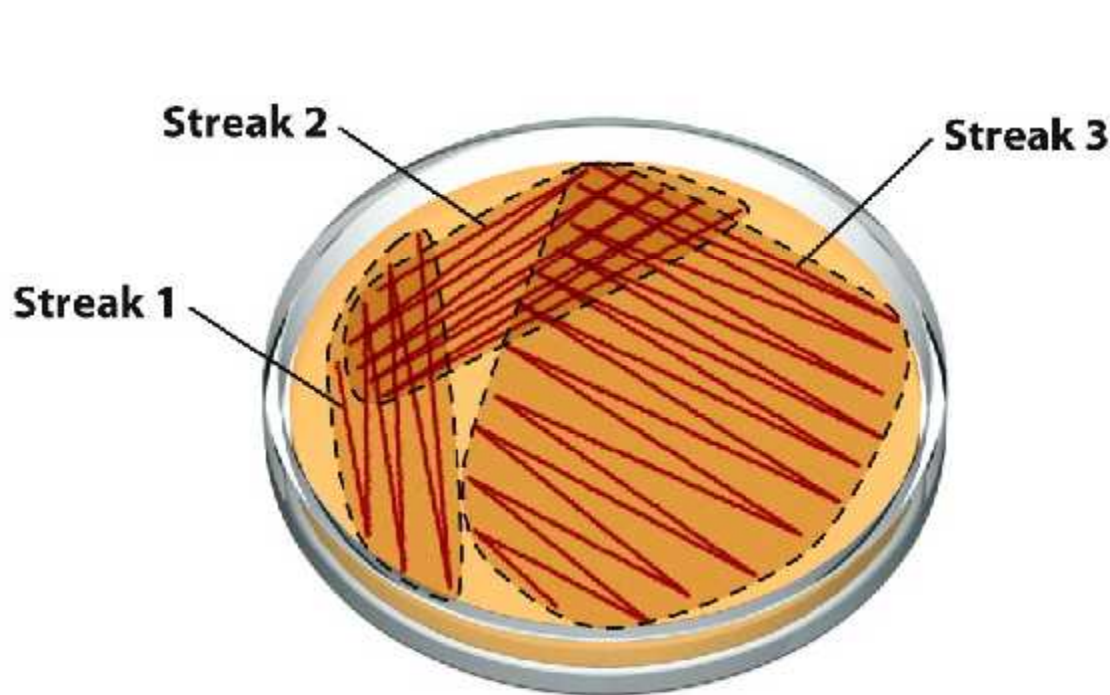
Streak 2

Streak 1

Streak 3

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Courtesy Christine Dupont

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A. Streak plate method for isolation of colonies

B. Isolated colonies after incubation

- Obtaining a pure culture: Spread/pour plate methods

0.1 mL

1 mL

Solid agar medium

Spread plate method

Incubation

Bacterial colonies on surface

Diluted bacterial suspension

9 mL of molten agar medium

Pour plate method

Incubation

Bacterial colonies on surface and in agar

18
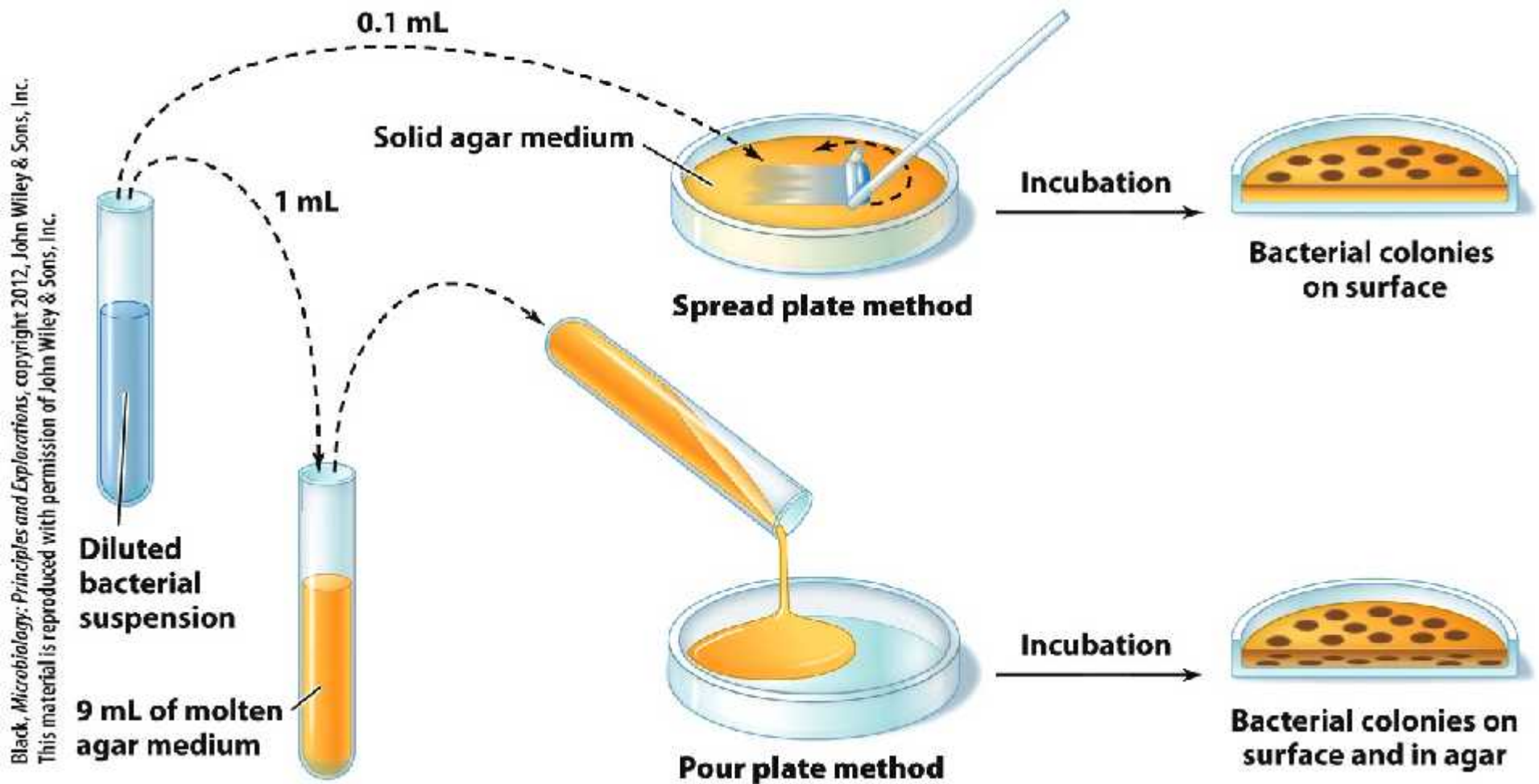
Growth of Microbial Populations

- Most unicellular microorganisms reproduce by *binary fission,* a process in which a cell grows to twice its normal size and divides in half to produce two daughter cells of equal size.
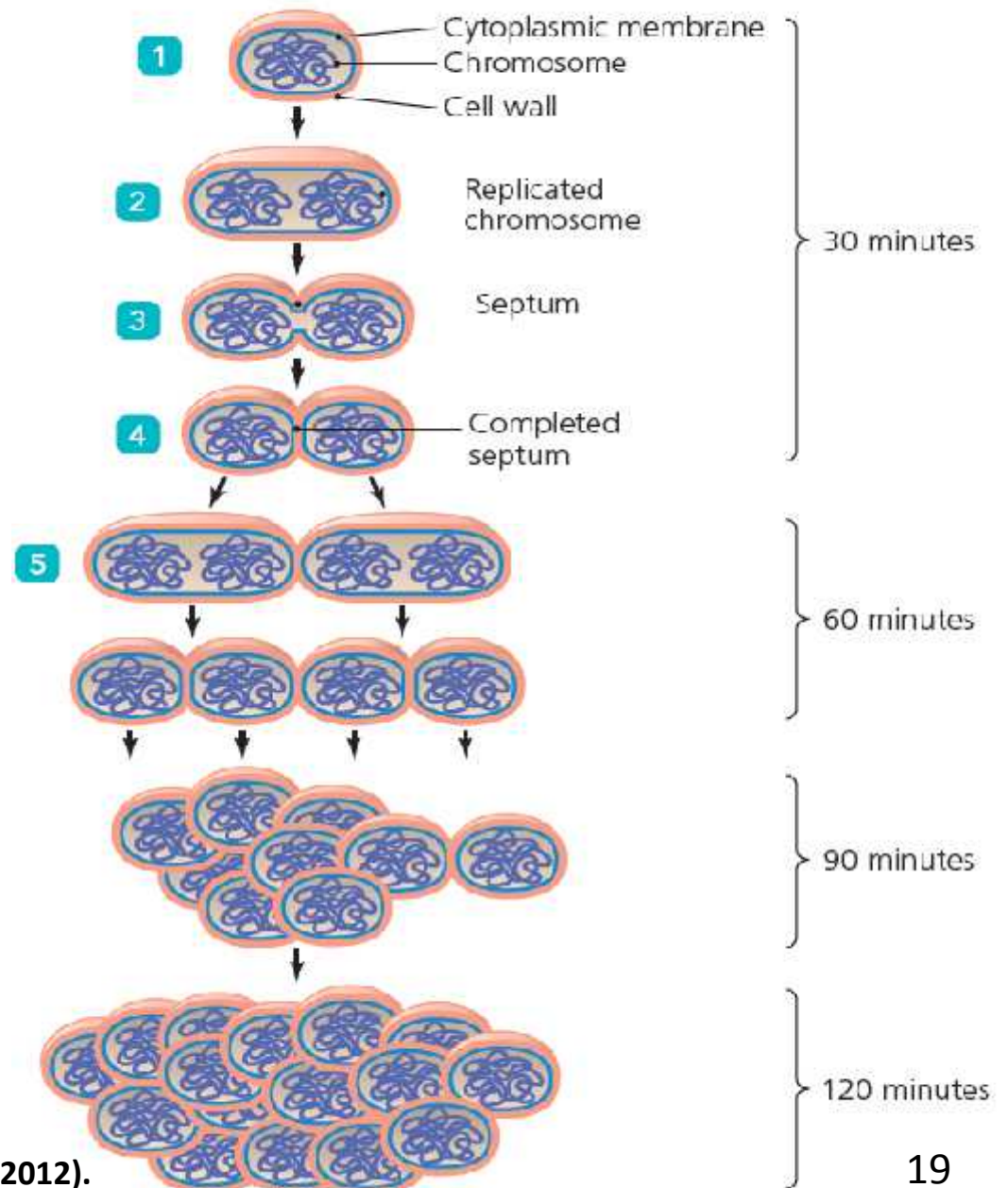
This Figure was taken from **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**

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Bacterial Cell Division
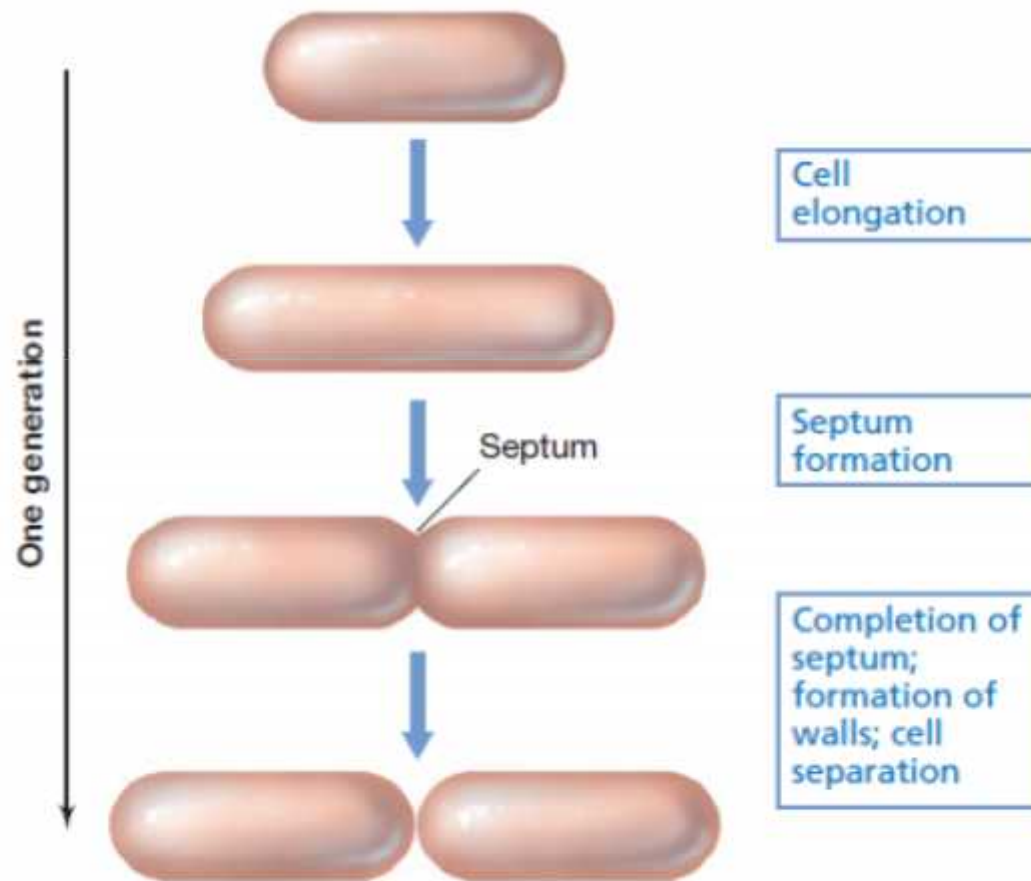
Figure 5.1 Binary fission in a rod-shaped prokaryote. Cell numbers double every generation.

Time (h)	Total number of cells
0	1
0.5	2
1	4
1.5	8
2	16
2.5	32
3	64
3.5	128
4	$256\ (2^8)$
4.5	$512\ (2^9)$
5	$1{,}024\ (2^{10})$
5.5	$2{,}048\ (2^{11})$
6	$4{,}096\ (2^{12})$
.	.
.	.
10	$1{,}048{,}576\ (2^{19})$

This Figure was taken from **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**
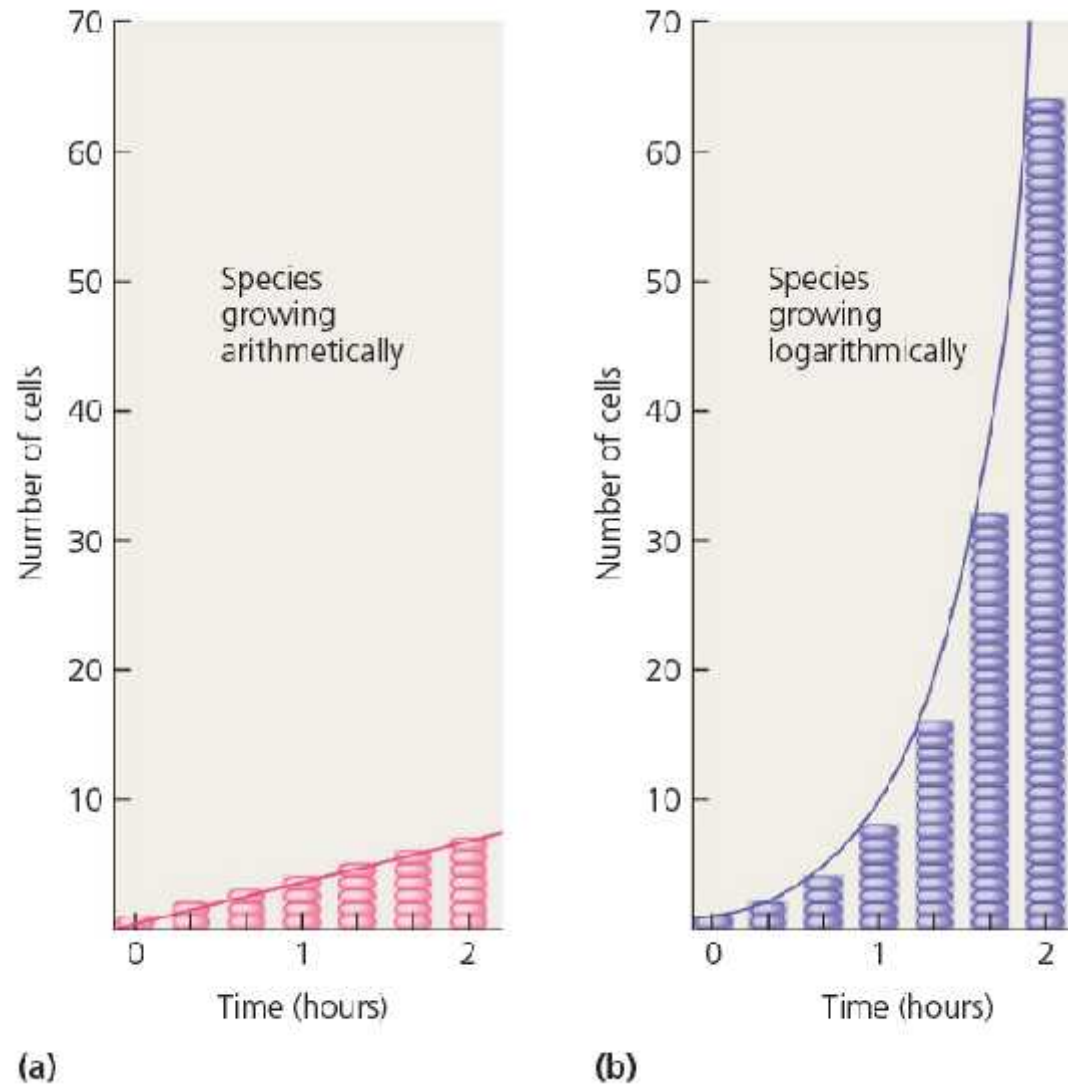
▲ **FIGURE 6.18** A comparison of arithmetic and logarithmic **growth.** Given two hypothetical initial populations consisting of a single cell each, after 2 hours an arithmetically growing species will have 7 cells, (a) whereas a logarithmically growing species will have 64 cells (b).

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Measuring microbial population growth:

- The microbial growth curve
  - A spectrophotometer can also be used to determine the four basic phases of a microbe's growth curve in a closed (or batch) culture over time.
    - Lag phase: Microbes are gearing up for steady growth.
    - Log phase: Microbes are replicating at a constant and steady exponential rate.
    - Stationary phase: Replication has either halted due to lack of nutrients and excessive wastes, or the rate of replication is now equal to the death rate.
    - Death phase: Nutrients are depleted, and waste levels are high; cells are dying at a steady exponential rate.

- The microbial growth curve

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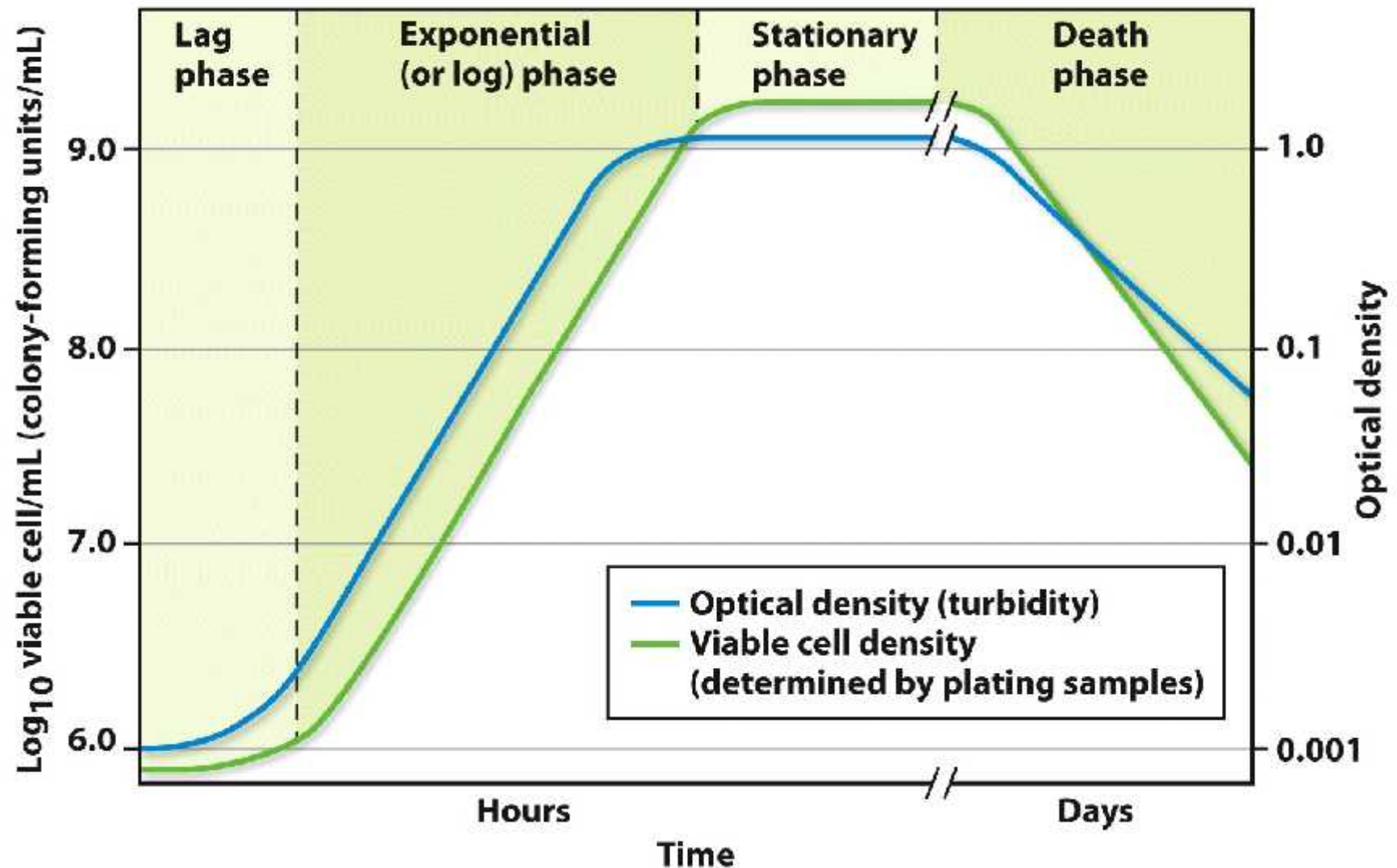
Measuring microbial population growth:

- The microbial growth curve
  - By analyzing the data from a growth curve, one can determine
    - Generation time: The time to double the population in the exponential phase
    - Growth rate: Number of generations/unit of time (inverse of the generation time)
    - Growth yield: The maximum population density and/or amount of cellular material produced by the culture

  Can you think of a situation where you would want to know each of these pieces of information?

Measuring microbial population growth:

- Continuous culture
  - Microbes in nature don't exist in a closed system.
    - We may want to keep microbes in exponential growth to harvest one of their products.
    - We may want to keep microbes in a limited but continuous flow of nutrients to mimic their environmental conditions.
  - A chemostat flows in fresh medium and takes out some old medium/microbes to keep the culture in continuous operation.

- Continuous culture
  - The human gut can be considered a continuous culture (see Perspective 6.3 for details).
    - Nutrients are continually added.
    - Waste and excess microbes are occasionally removed.

Fresh medium — Control valve — Pump — Culture vessel — Sampling port — Air supply — Collection vessel

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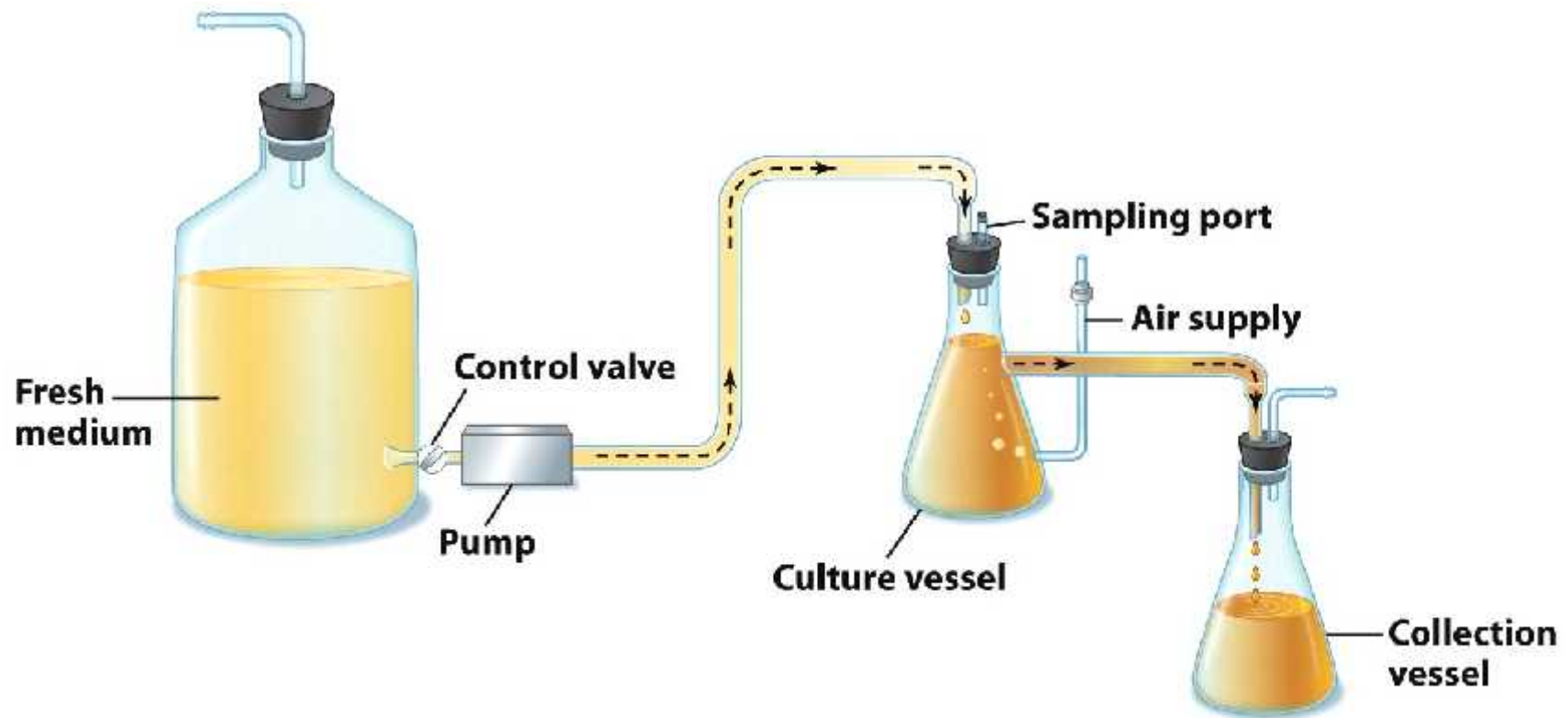
Measuring microbial population growth:

- *How can we measure and count microbes?*
  - Different methods exist, including:
    - direct counts
    - viable (living) cell counting
    - turbidity (cloudiness) measurements

Measuring microbial population growth:

- Direct counts
  - A special slide with an etched grid can be used.
  - A known volume is loaded onto the grid and cells are counted under a light microscope.
  - Pros: Cheap, fast, easy
  - Cons: You can't differentiate living vs. dead cells.

① Add a drop or two of bacterial suspension to the center of the chamber.

Bacterial suspension

Petroff–Hausser counting chamber

② Lower a cover slip over the grid. The suspension spreads out evenly and excess flows into the side channels.

Cover slip

Bacterial suspension

③ Use a microscope to count the cells in the grid.

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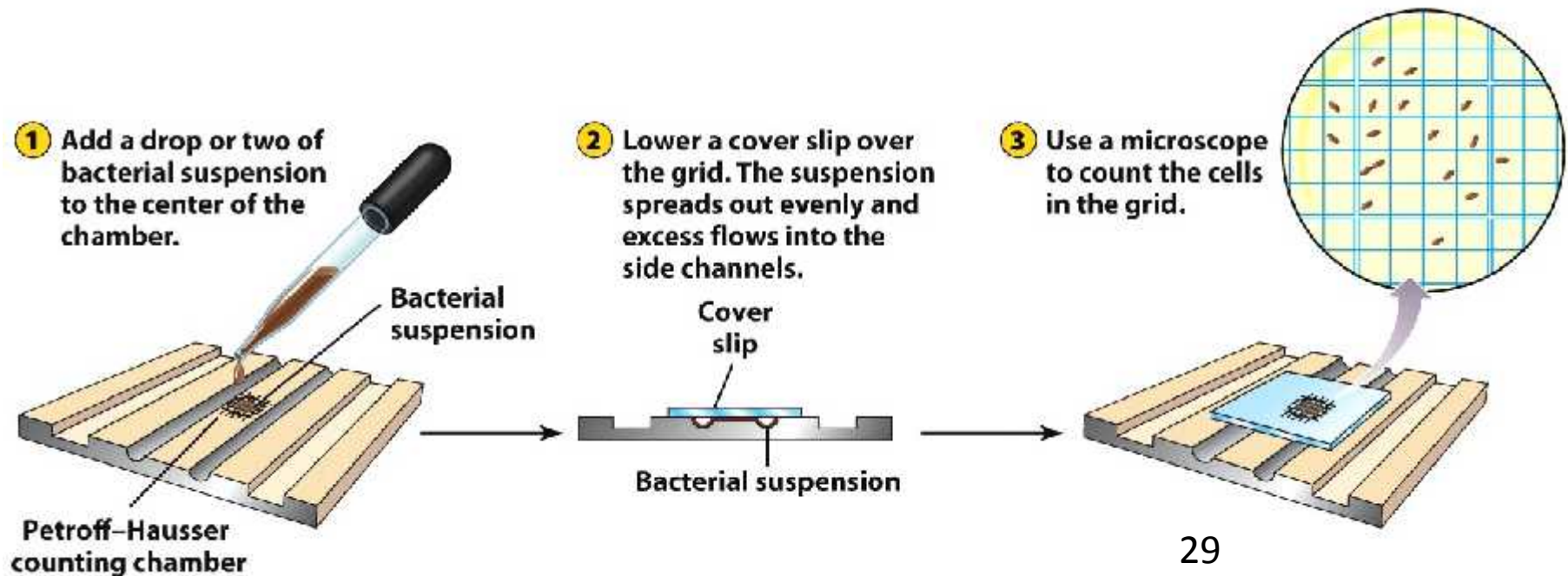
Measuring microbial population growth:

- Viable (living) cell counting – Serial dilutions and CFUs
    - First, the culture is diluted in a series of tubes.
    - The dilutions are plated (the spread plate method).
    - After incubation, colonies are counted.
    - Colony-forming units (CFUs) per milliliter of initial culture is calculated by multiplying the number of colonies by the inverse of the dilution factor.
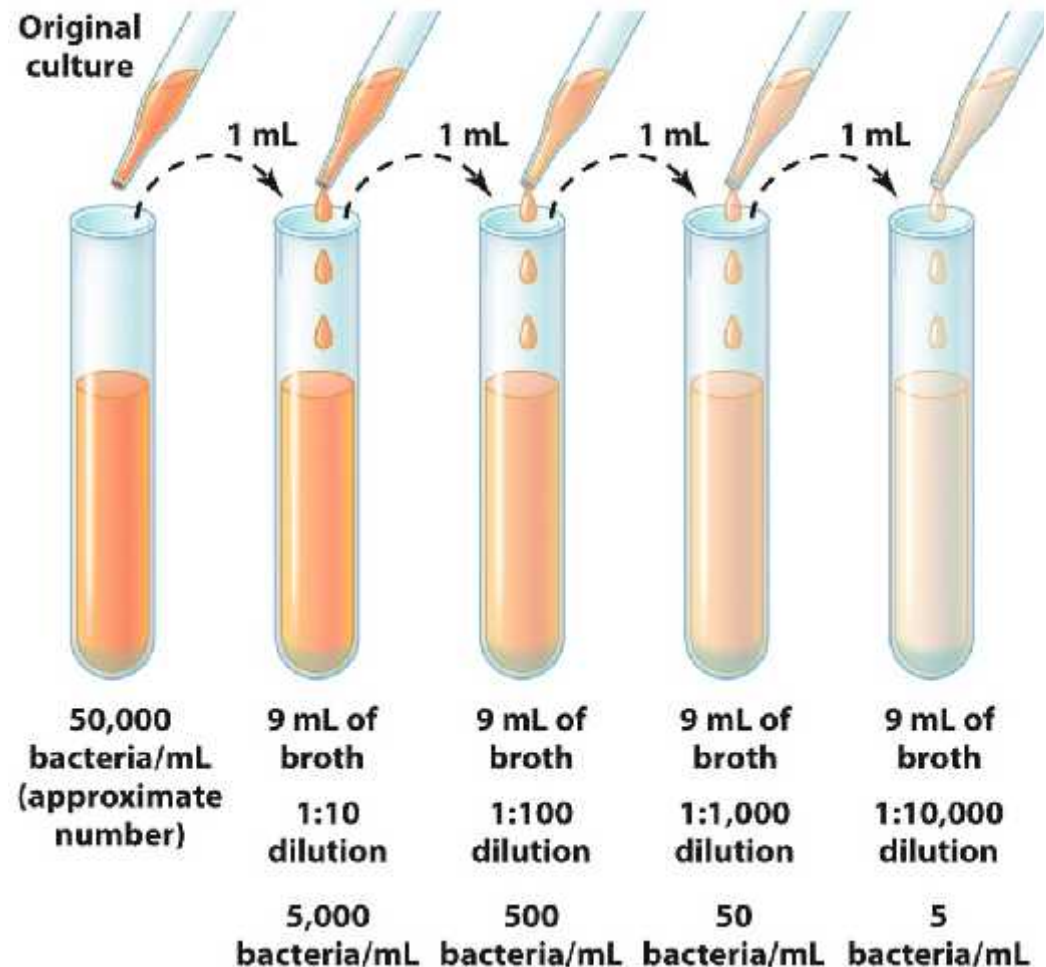
Measuring microbial population growth:

- Viable cell counting: Serial dilutions and CFUs
  - What if the cells are already very diluted?
  - A filter apparatus can concentrate the cells.

Sample

Membrane filter

Filter support

Transfer membrane to growth medium

To vacuum

Colonies

Incubate

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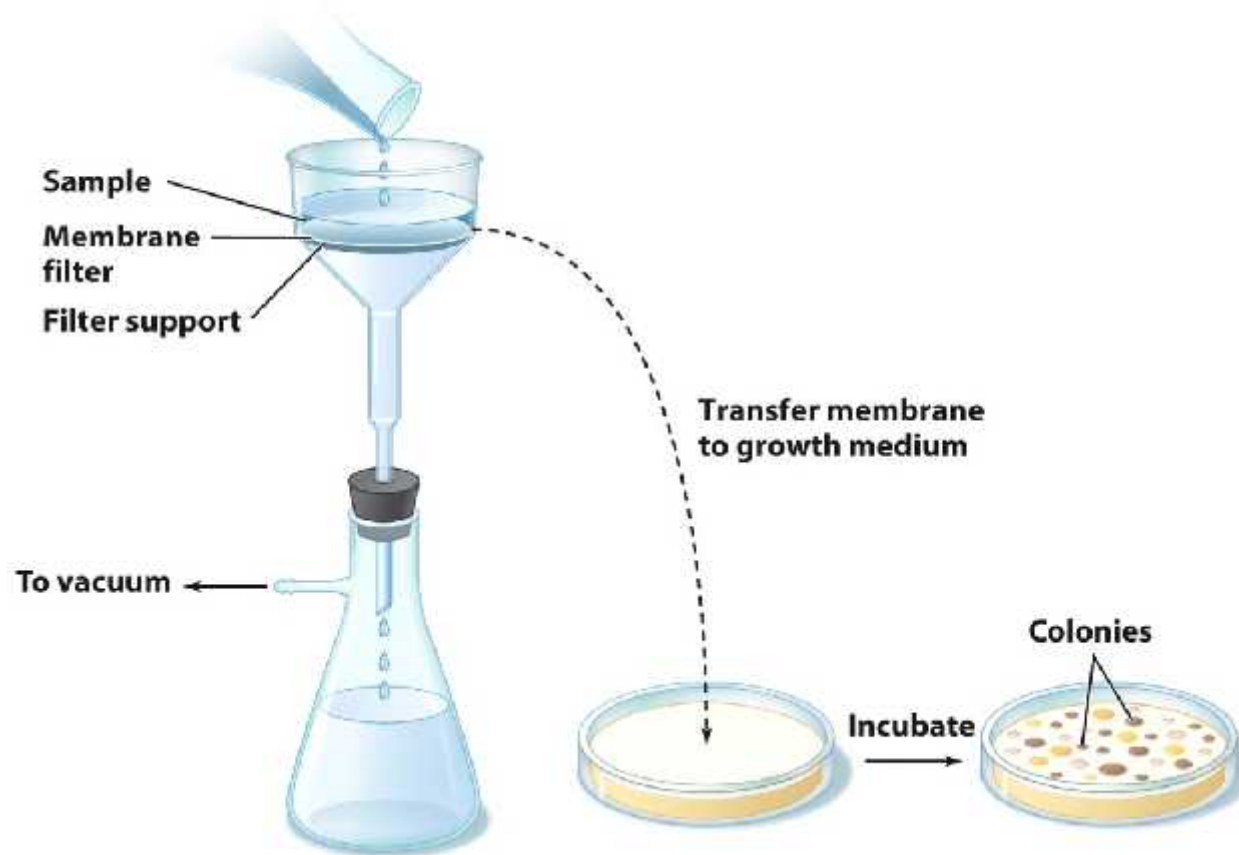
Measuring microbial population growth:

- Turbidity
  - A spectrophotometer sends light through a culture.
  - If the tube is cloudy, light won't get through the tube and strike the unit's sensor (high absorbance).
  - It can give a rough measure of cell density in the tube.
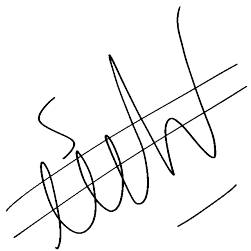
Light source   Sample receptacle   Uninoculated control solution   Spectrophotometer

Courtesy Christine Dupont

TRANSMITTANCE
ABSORBANCE
Percent light transmitted

Light-sensitive detector

A. Light transmission for uninoculated culture media

Bacterial suspension

Courtesy Christine Dupont

TRANSMITTANCE
ABSORBANCE
Percent light transmitted

Scattered light that does not reach detector

B. Light transmission for a bacterial culture

Microbiology for Nursing students

Chapter Three A: Innate Host Defenses Against Microbial Invasion

MICROBIOLOGY

Wessner • Dupont • Charles
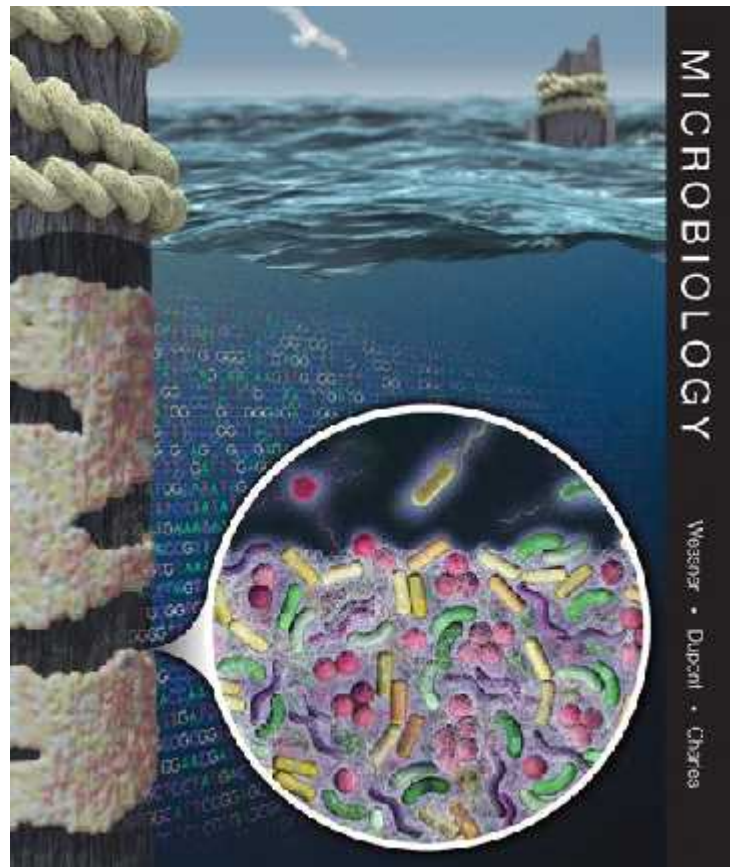
Dr. Sulaiman Alnaimat 2015

Introduction:

- Immunity is important to life.
  - We are constantly under assault from microbes in us, on us, and around us.
  - We must have systems in place to provide defense against this assault (which comprise immunity).
  - Immunity can be split into two parts:
    - Innate (the topic of this chapter)
    - Adaptive (the topic of the next section)

Immunity:

- *Are there different types of immunity?*
  - Immunology is the study of the components and processes of the immune system.
    - The immune system distinguishes foreign substances from self structures.
    - Vertebrates possess two systems:
      - Innate (non-specific)
      - Adaptive (specific)
    - These two systems work together to defend the body against foreign agents and cancerous cells.

Immunity:

- Innate immune defenses
  - Found in all multicellular organisms
  - Provide a first line of defense against microbes
  - Usually recognize biochemical differences between microbes and host cells
  - While microbes can be recognized as "foreign," this system can't discern the precise identity of the microbe.
    - It simply responds to an entire group of similar microbes in the same manner.
    - It is therefore "nonspecific" in the nature of its responses.

Immunity:

- Adaptive immune defense (discussed in the next chapter!)
  - Found only in vertebrates
  - Works with innate responses to achieve a stronger level of defense
    - However, it recognizes specific pathogens rather than broad classes of microbes.
    - The response is mediated by molecules that bind to specific pathogens.
  - After initial exposure, it retains memory of the response used and can initiate it more quickly (and more effectively) upon re-exposure.
    - Hence the term "adaptive" immunity—it gets better with each exposure.

Barriers to infection:

- *What are the built-in barriers to infection?*
  - Skin
    - Generally inhospitable to foreign microbes
      - Cool, dry, acidic (pH 5.0)
      - Dead layer of cells on top provides an "armor."
      - A layer of antimicrobial oil (sebum) lies on top of this layer.
      - Sweat secretions can also provide an antimicrobial barrier.
    - Normal flora microbes colonize the skin and can "crowd out/starve out" potential invaders.

Sloughing removes adherent microbes.   Hair shaft

Hair follicle
Sebaceous gland
Sweat gland
Epidermis
Dermis

Keratinized dead cells form a protective barrier.

Tight cell junctions prevent microbial invasion.

Sweat glands secrete antimicrobial substances, such as NaCl and lysozyme.

Sebaceous glands secrete toxic fatty acids.
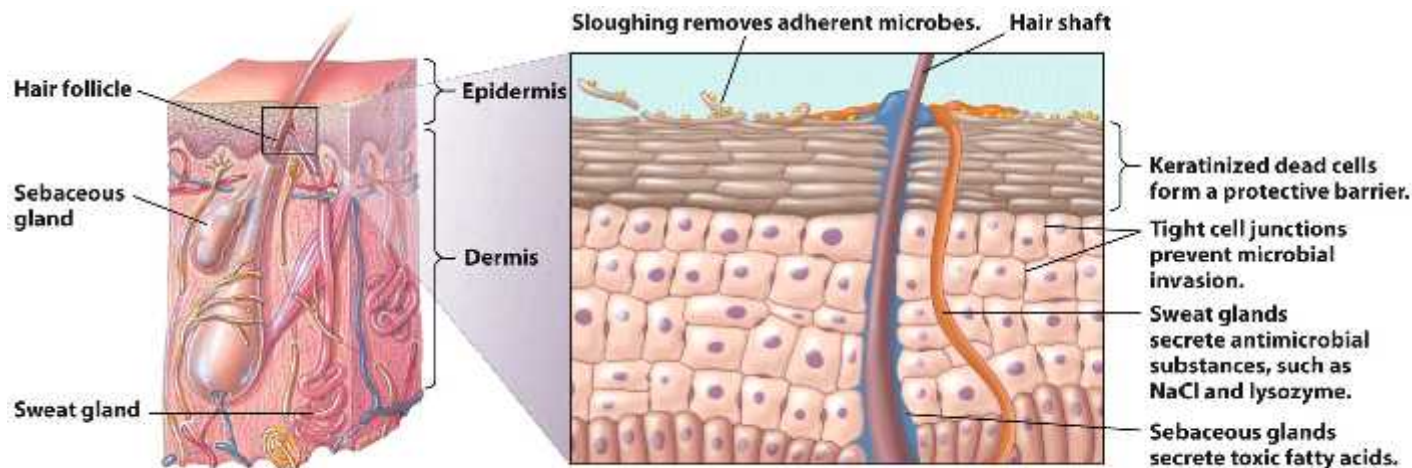
Barriers to infection:

- Mucosal membranes
  - Interior surfaces coated with wet mucus
    - Moved along the surface to prevent microbe attachment
    - Contain antimicrobial molecules
      - Defensin proteins/ Lysozyme/ Lactoferrin
  - Competitive exclusion by normal flora (again!)

Mucus traps microbes.

Cilia sweep mucus out of respiratory tract.

Tight junctions prevent microbial movement to underlying tissue.

Ciliated epithelium

Goblet cell secretes mucus.
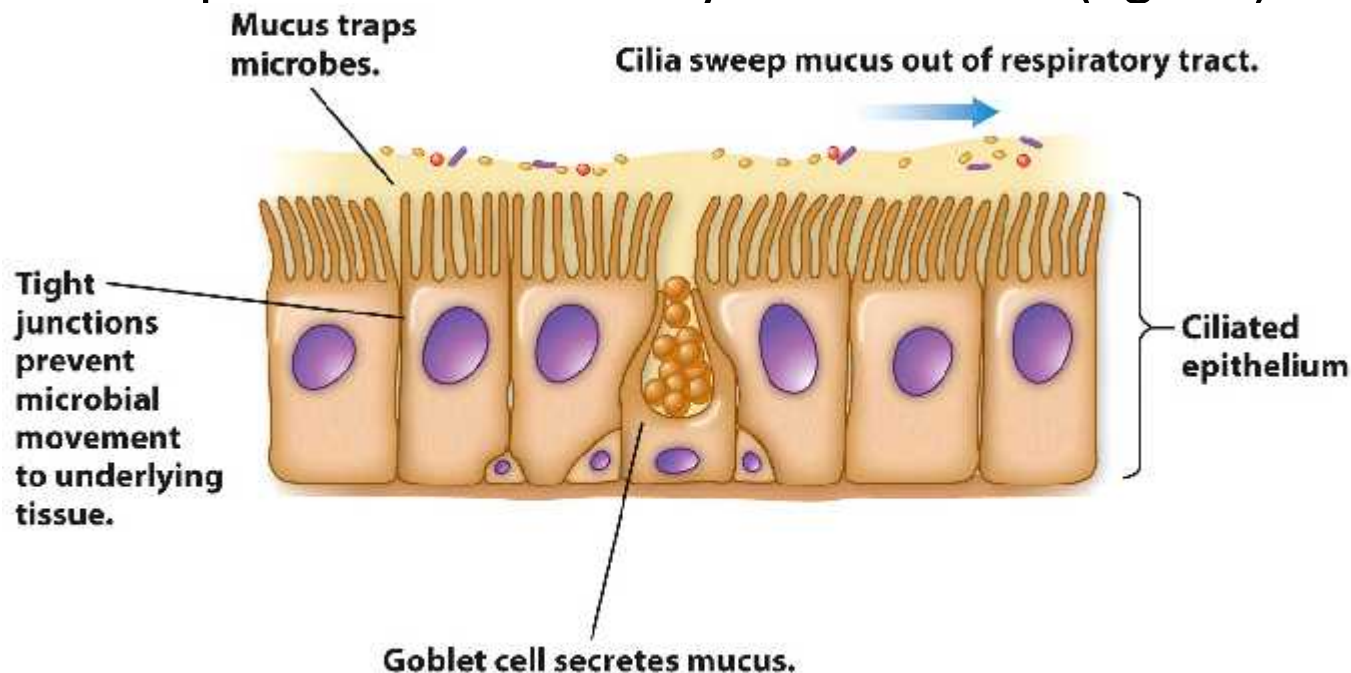
Ciliated mucosal surface of upper respiratory tract

Mucus globules

Goblet cells

Lumen of intestine

Nuclei of epithelial cells

TEM © Steve Gschmeissner/ Photo Researchers, Inc.

LM © Innerspace Imaging/ Photo Researchers, Inc.
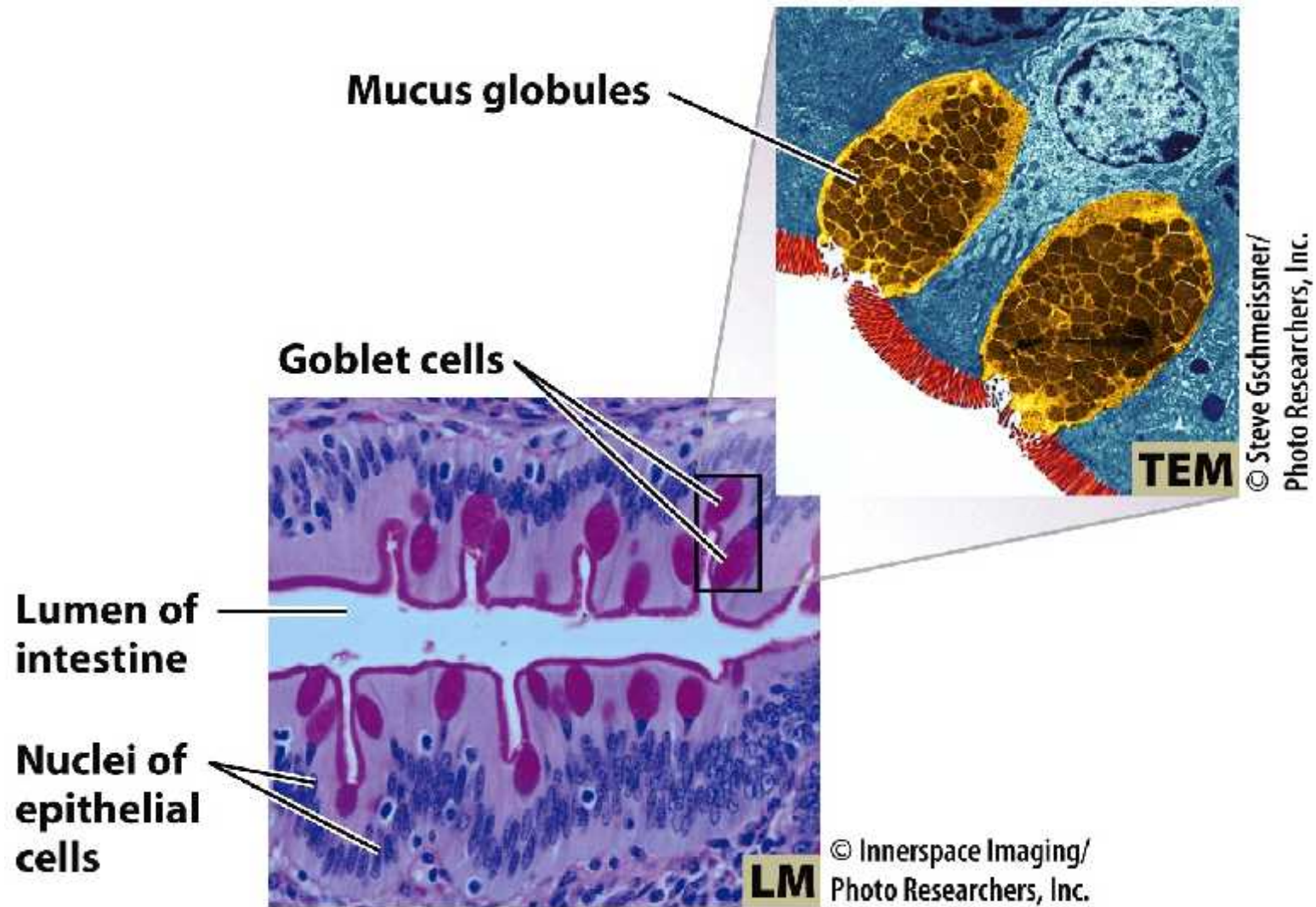
Mucosal surface of small intestine

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TABLE 19.1 Physical and chemical innate defenses associated with mucosal membranes

Site	Mucosal surface	Defense
Eye	Conjunctiva	Filtering by eyelashes
		Lysozyme
		NaCl
		Flushing by tears
Digestive tract	Mouth	Lysozyme
		Antimicrobial peptides
		Desquamation
		Mucus
	Stomach	Mucus
		pH 2–3
	Small intestine	Peristalsis
		Digestive enzymes
		Bile salts
		Desquamation
		Mucus
		Antimicrobial peptides

TABLE 19.1 Physical and chemical innate defenses associated with mucosal membranes

Site	Mucosal surface	Defense
	Colon	Large numbers of resident microbiota
		Mucus
		Peristalsis
Respiratory tract	Nasal passage	Filtering by nostril hairs
		Mucus
	Trachea and bronchi	Ciliated epithelium
		Mucus
	Lungs	Lactoferrin
		Lysozyme
		Antimicrobial peptides
Urogenital tract	Urethra	Flushing by urine
	Vagina	Antimicrobial peptides
		pH 5
		Mucus
		Ciliated epithelium

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- **Iron: The limiting element**
  - Keeping iron for OUR cells is important.
    - Microbes need iron to grow.
    - We need iron for our own cells' function.
    - Several cell types make molecules to keep iron away from microbes.

TABLE 19.2	Iron-binding molecules of the body	
Iron-binding protein	**Location**	**Function**
Hemoglobin	Red blood cells	Binds oxygen
Myoglobin	Muscle and heart cells	Binds oxygen
Transferrin	Plasma	Transport of iron around the body
Lactoferrin	Milk, tears, saliva	Antimicrobial activity in secretions by binding iron
Ferritin	Cells, small amounts present in plasma	Storage of iron

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The inflammatory response:

- What is an early response of the body to infection?
  - Inflammation = Important, early physiologic response to microbial invasion and damage
    - Triggered by release of proinflammatory molecules (such as histamine and cytokines) from local cells
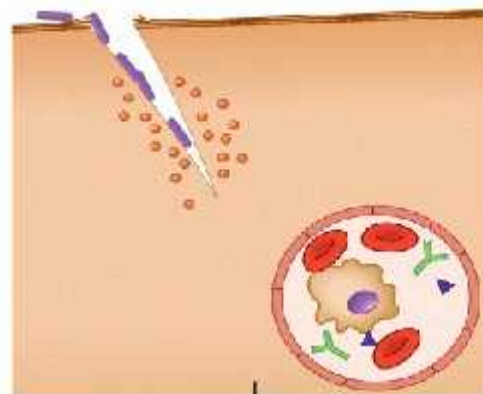
1. Bacteria enter the tissue through a cut.
2. Vasodilator molecules are released such as histamine, prostaglandins, and serotonin.

Blood vessel

Legend:
- Bacteria
- Phagocyte
- Antibody
- Complement factor
- Red blood cell
- Vasodilator
- Cytokine
- Adhesion molecules
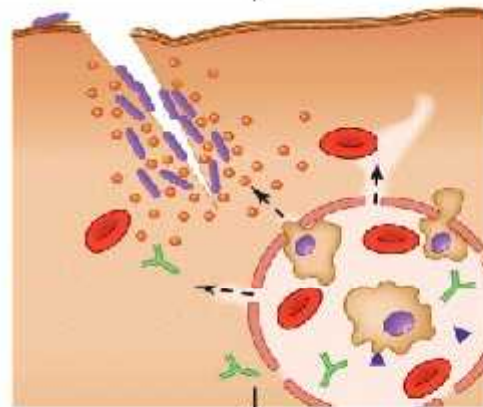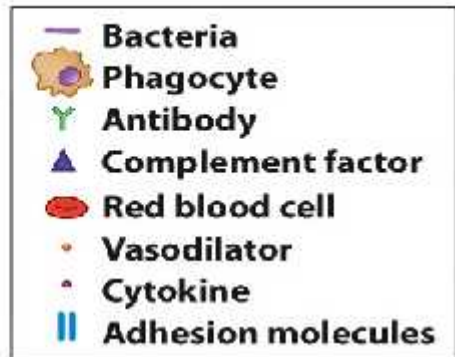
3. Bacteria multiply and invade tissue.

4. Concentrations of vasodilators increase.

5. Vessel dilates, allowing more blood into the area. An increase in local temperature and redness occurs.

6. Vessel walls become more permeable. Fluid moves into the tissue, causing swelling. Extravasation of cells and molecules occurs.
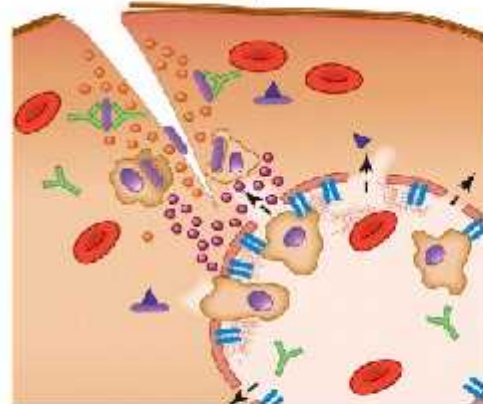
7. Antibody and complement factors bind the bacteria.
8. Phagocytes engulf and destroy the bacteria. Cytokines are released, attracting more leukocytes.

9. Adhesion molecules are expressed on vessel walls to facilitate extravasation of immune cells.

10. Clotting is initiated to restrict access of bacteria to circulatory system.

The inflammatory process

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The inflammatory response:

- Consequences of local inflammation
  - Vasodilation
  - Extravasation
  - Increase in vessel permeability
- These bring in immune cells (e.g., phagocytes) to fight the infection.
- Cytokines are often used to communicate the status of an infection.
  - Producing fever (via IL-1, IL-6, TNF-α)
  - Enhancing inflammation (via IL-8)
  - Stimulating further immune responses

The inflammatory response:

- Consequences of systemic inflammation
  - If inflammation gets out of hand, it can be damaging.
  - This can happen when microbes or their products get into the bloodstream.
    - Septic shock: Widespread presence of bacteria in the body induces system-wide inflammation.
    - Toxic shock: Overstimulation of immune responses by bacterial exotoxins in the blood
  - Death rates of 30–50% are not uncommon when septic or toxic shock sets in.

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The molecules of the innate system:

- How do we sense or detect the presence of pathogens?
- Pathogen-associated molecular patterns
  - How does the innate system figure out what is foreign and what isn't?
  - PAMPs = Pathogen-associated molecular patterns
    - Structures found on foreign microbes but NOT on self cells
      - PRRs = Pattern recognition receptors
        - » Receptors on our cells that can bind to PAMPs to begin the responses against them
        - » Don't recognize molecules on individual pathogens but rather common molecules found on entire groups of pathogens (nonspecific)

The molecules of the innate system:

- Toll-like receptors (TLRs)
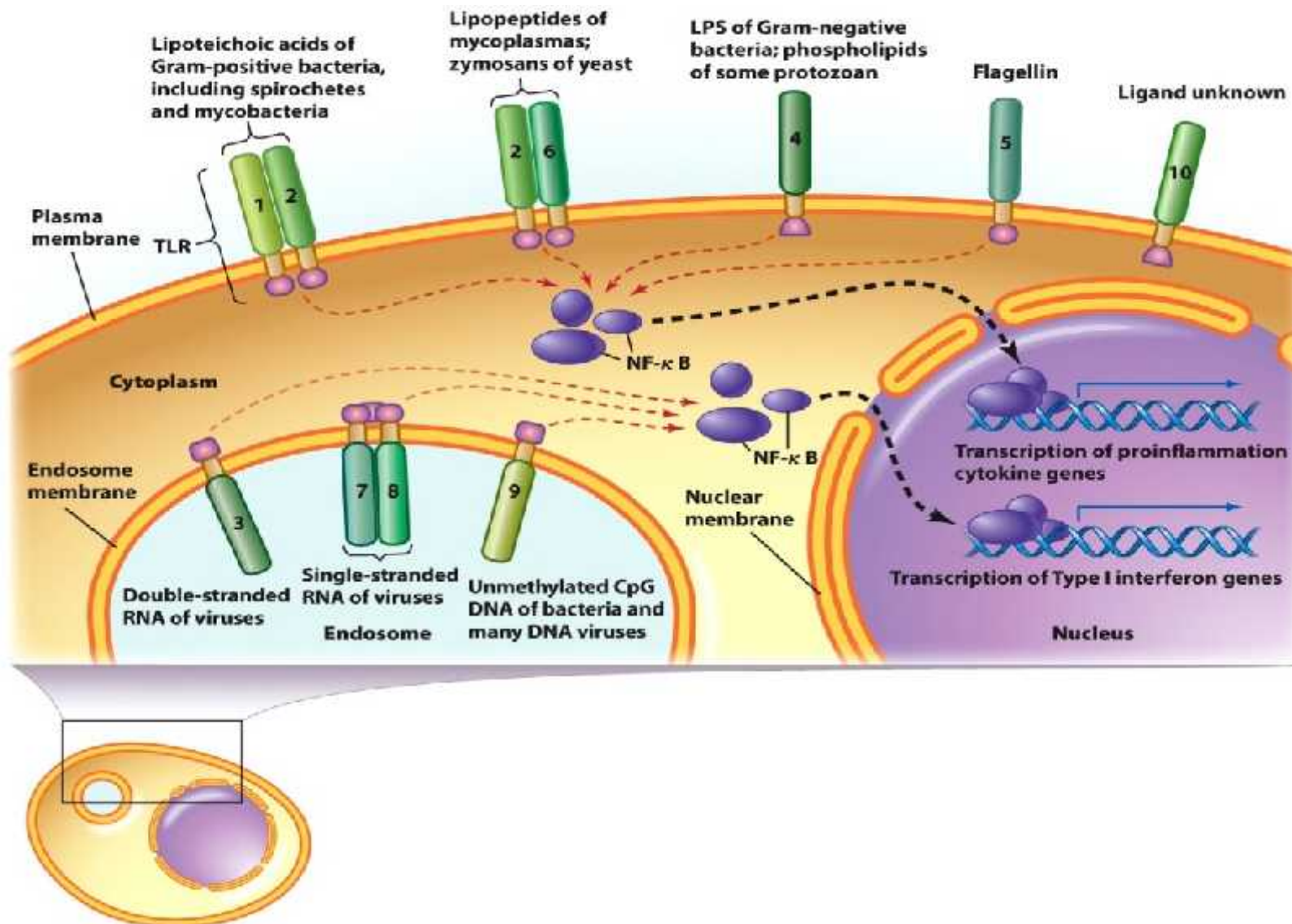
The molecules of the innate system:

- Toll-like receptors (TLRs)
  - Different TLRs can detect different PAMPs.

  - Different TLRs may produce different cytokine responses for maximum effectiveness.
    - TLR4 binding of LPS stimulates production of IL-1 and TNF-α.
      - These cytokines initiate a strong antibacterial response.

The molecules of the innate system:

- Mannose-binding lectin and C-reactive protein
  - These are examples of opsonizing-secreted PRRs.
    - Opsonization = Coating of a microbe, enhancing destruction or uptake by other cells
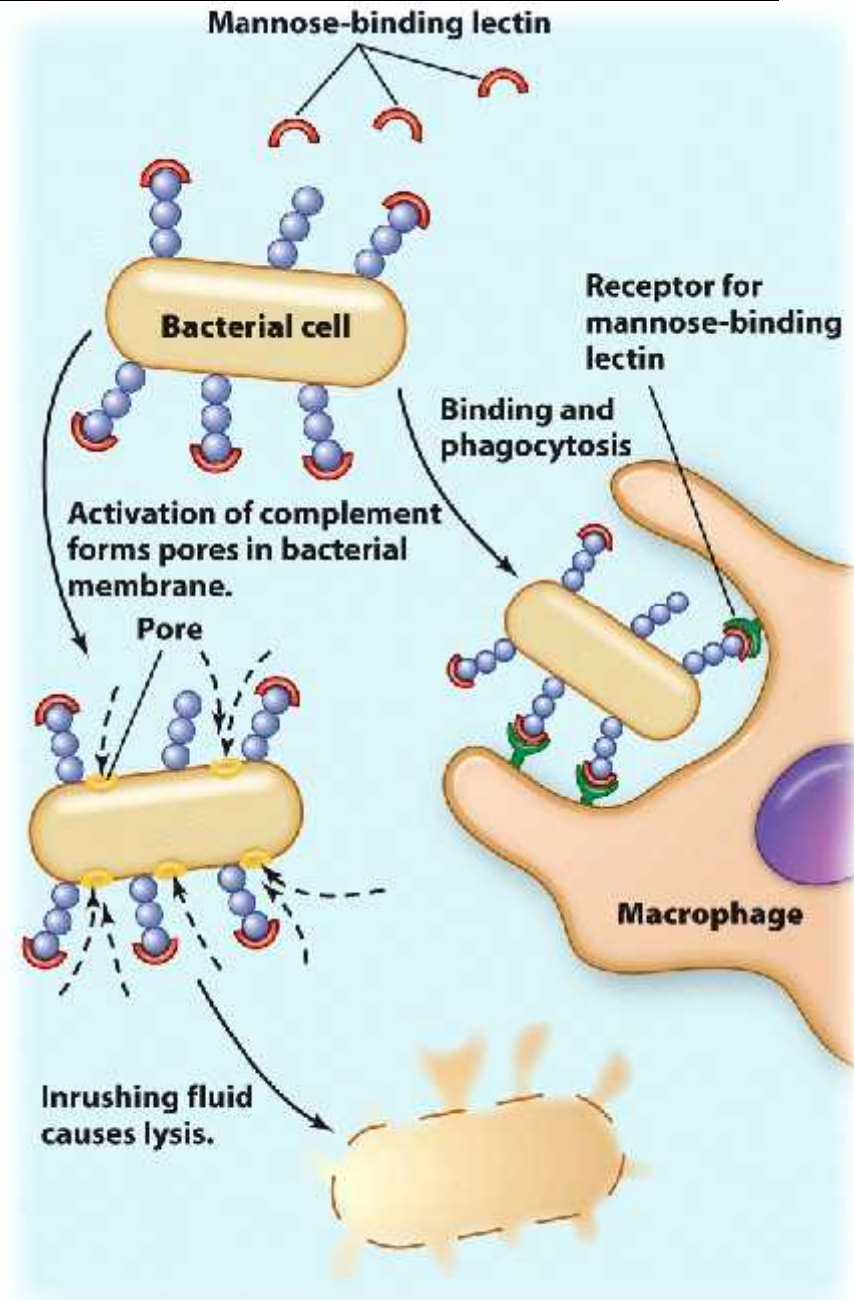
The molecules of the innate system:

- Complement
  - A group of 30+ serum proteins involved in antimicrobial activities
  - Nine particular complement proteins become activated in a cascade in the presence of PAMPs.
  - Activation results in
    - Inflammation
    - Opsonization (enhancing phagocytosis)
    - Direct microbe killing by formation of the membrane-attack complex (MAC)

The molecules of the innate system:

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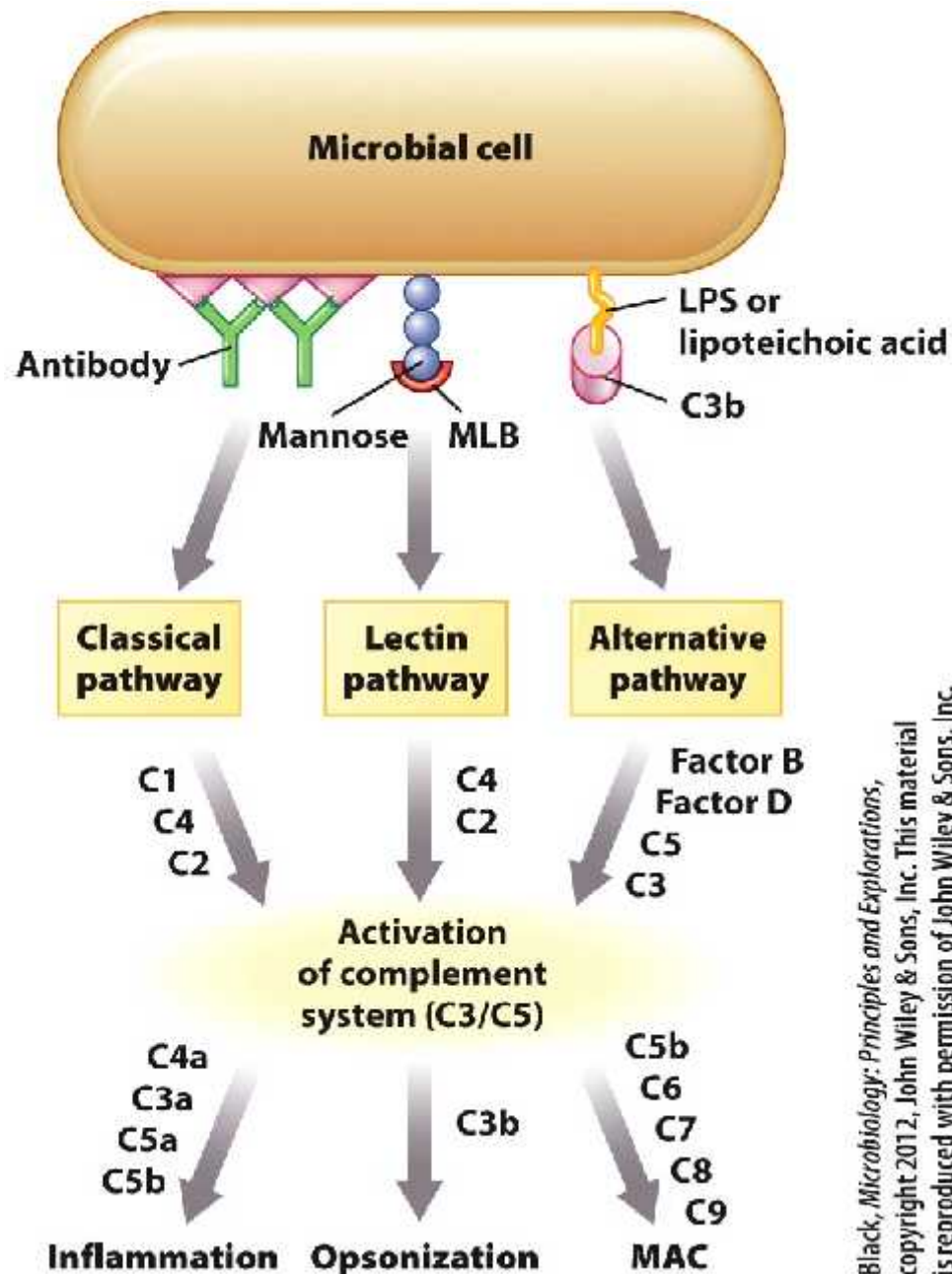
Complement
- Three separate methods of activating the complement cascade exist.

The molecules of the innate system:

- Complement: Opsonization/phagocytosis
  - Another function of complement
  - Coating with activated complement increases chances for phagocytosis

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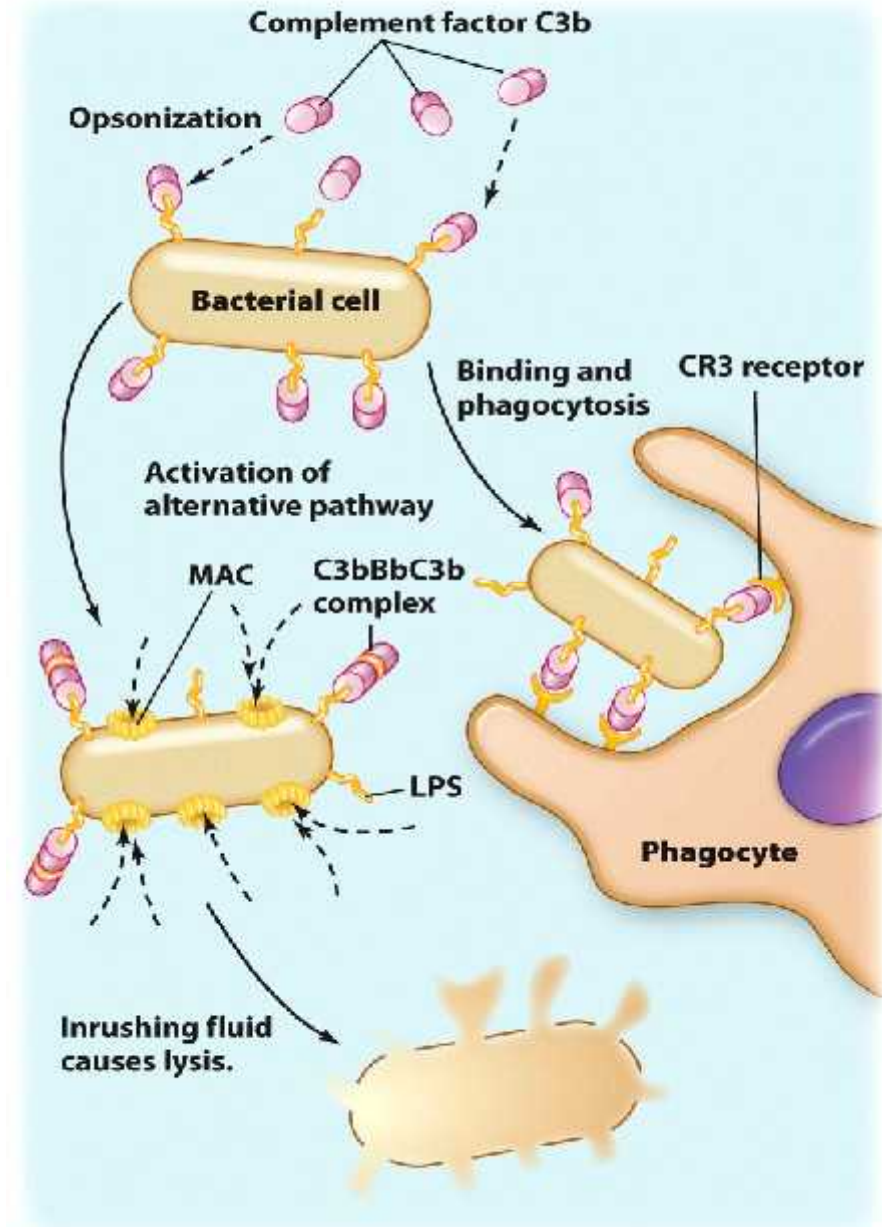
The cells of innate immunity:

- What cells first defend us against infectious agents?
  - Phagocytes
    - Immune system cells that engulf foreign invaders
      - Include neutrophils, monocytes, and macrophages
    - Activation through PRRs and cytokine signaling turn the cells into efficient killing machines.

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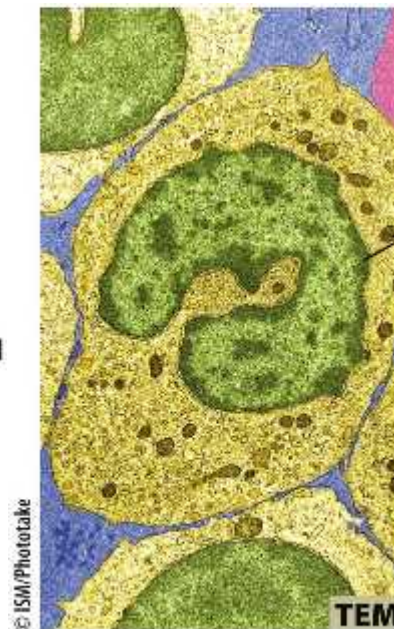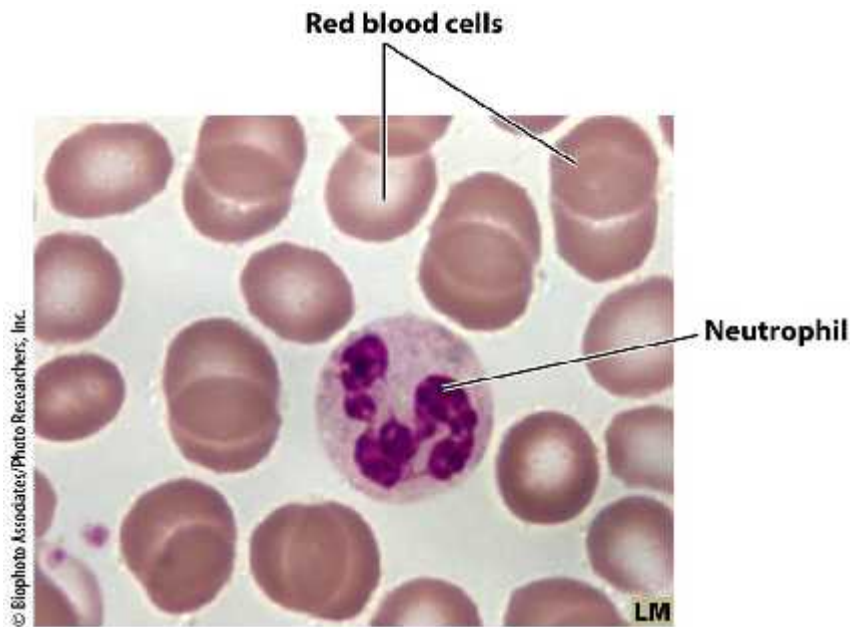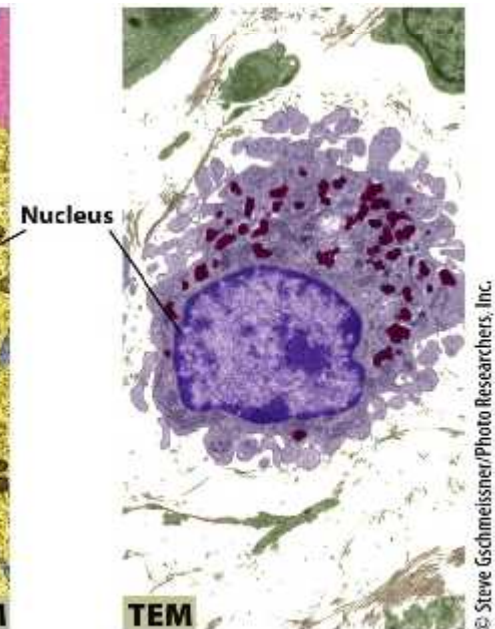
The cells of innate immunity:

- Phagocytes
  - Opsonization prior to ingestion enhances uptake.
  - Neutrophil granule contents released extracellularly attack invaders.
  - Once the invader is ingested, a complex process takes place to destroy it.
    - Often this process involves fusion with lysosomes and the use of a controlled respiratory burst.

Lipopolysaccharide (LPS) of Gram-negative bacteria, or lipoteichoic acid (LTA) of Gram-positive bacteria

Surface antigen

Mannose units

Phosphocholine

Bacterial cell

CR3 receptor

FcR receptor binds IgG antibody and C-reactive protein

Phagocyte plasma membrane

Receptor for mannose-binding lectin

Cytoplasm

Complement factor C3b

Antibody

Mannose-binding lectin

C-reactive protein

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The cells of innate immunity:

① Opsonized microbe binds to phagocyte surface receptors.

Opsonin
Microbe
Receptor
Neutrophil or macrophage
Lysosomes containing digestive enzymes

② Cytoplasmic extensions surround and engulf the bound microbe.

Don W. Fawcett/Photo Researchers, Inc.

TEM

③ Phagosome forms and acidification begins.

Phagosome

H⁺

④ The phagosome fuses with lysosomes to form a phagolysosome.

Phagolysosome

⑤ Oxidative burst and lysosomal enzymes kill the microbe.

⑥ Exocytosis expels the debris.

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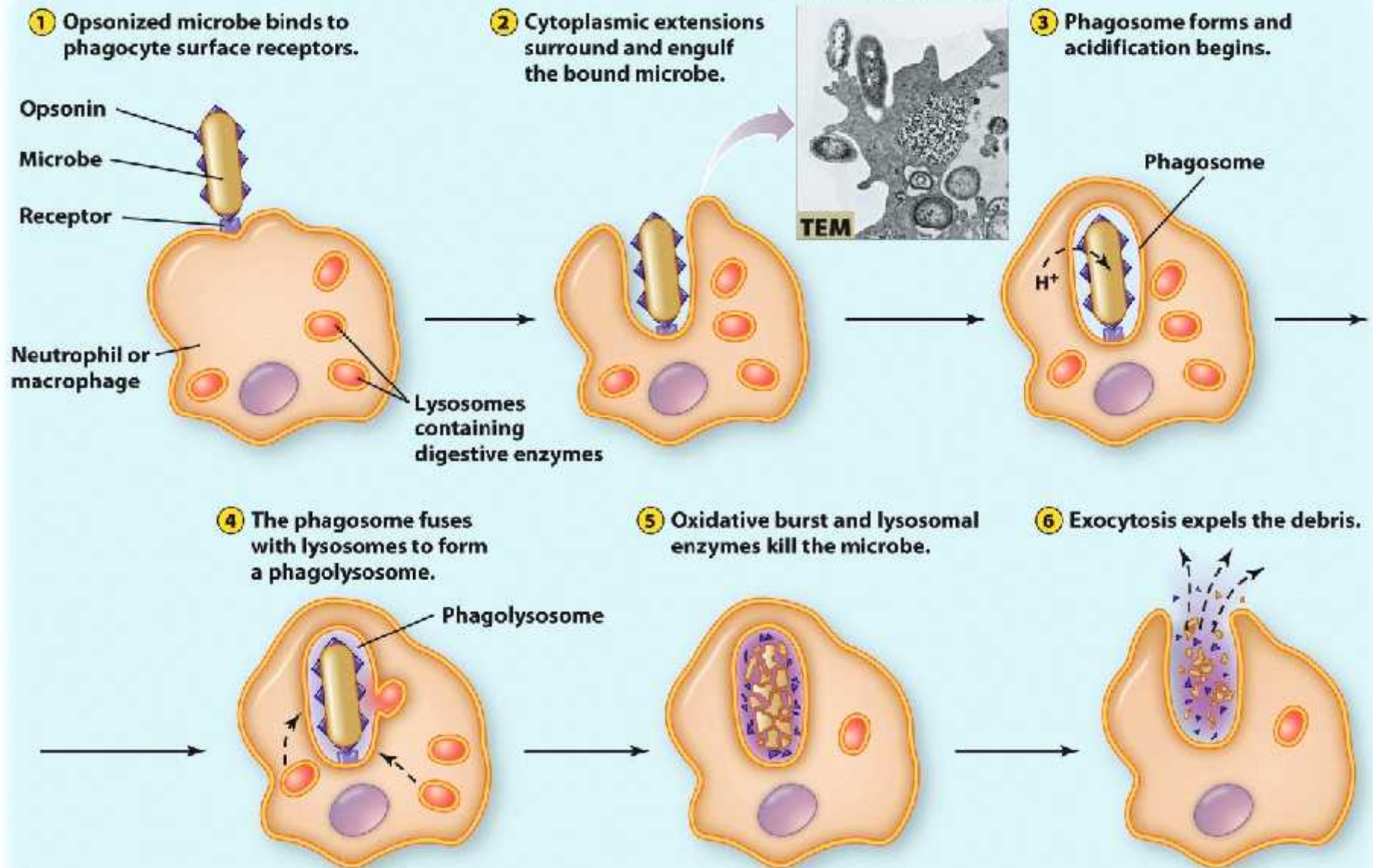
The cells of innate immunity:

TABLE 19.6 Antimicrobial compounds of lysosomes and their effects

Molecule	Killing action
Lysozyme	Hydrolysis of peptidoglycan of the bacterial cell wall
Proteases	Degradation of proteins
Defensins	Form pores in bacterial membranes

TABLE 19.7 Toxic oxygen products of the respiratory burst

Chemical formula	Common name
H_2O_2	Hydrogen peroxide
HOCl	Hypochlorite
OH	Hydroxyl radical
O_2	Superoxide anion
NO	Nitric oxide

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The cells of innate immunity:

- Eosinophils, basophils, and mast cells
  - Some pathogens are too big for phagocytosis.
    - For example, extracellular parasitic worms, certain fungi
  - These cells help fight such pathogens, releasing toxic granule contents (degranulation process) near them.

The cells of innate immunity:

- Natural killer (NK) cells
  - Useful for eliminating host cells infected with pathogens (kill one ill cell, save many healthy cells)
  - Not phagocytic but DO make contact with target cells
    - After contact is initiated, granule components are released.
      - Perforin produces a pore structure in target cell plasma membrane.
      - Granzymes induce apoptosis (controlled cell suicide).
  - Also useful for eliminating abnormal self cells (cancer)

- # Natural killer (NK) cells: How are apoptosis and necrosis different?

Apoptosis

1a Cells signaling occurs.

2a The cell decreases in volume.

3a Cytoplasmic blebbing and chromatin condensation begin.

Chromatin

4a DNA fragmentation occurs and the cell is parceled into apoptotic bodies.

5a A phagocytic cell digests the apoptotic bodies.

No inflammation

Normal cell

Necrosis

1b Physical or chemical trauma occurs.

2b The cell membrane is damaged.

3b The contents of the cell leak into the surrounding tissues.

4b Nearby cells may be damaged by the released cell contents.

Inflammation

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The cells of innate immunity:

- Natural killer (NK) cells
  - How do they recognize an abnormal cell from a normal one?
    - Normal nucleated cells have a surface molecule known as "class I major histocompatibility complex" (MHC I).
    - NK cells recognize targets that lack this molecule.
      - Virally-infected cells often turn off its expression.
      - Cancer cells tend to shut down expression as well.
    - But the story (and the recognition capabilities) are more complex than that (see next slide)!

Neutrophil

F_CR MHC I CR3

T cell

MHC I

T-cell receptor

CD4 or CD8

CD3

CD2

NK cell

MHC I

CD2

CD56

CD94

B cell

CD19

CD35

CD20

B-cell receptor

MHC II

MHC I

CD40

MHC I

F_CR

MHC II

CR3

CD86

Epithelial cell

MHC I

Macrophage or dendritic cell

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- Natural killer (NK) cells

NK cell

Inhibitory signal

Normal/uninfected cell

Activation + Inhibitory signal signal = no killing

Granules containing perforin and granzyme

Activation signal

A. Normal MHC I expression on cell surface

NK cell

No inhibitory signal

Abnormal/infected cell

Activation Inhibitory signal − signal = killing

Release of perforin and granzyme

Apoptotic bodies will be phagocytosed and destroyed.

Apoptosis

Activation signal

CD94

MHC I receptor

Activating ligand

Activating receptor

Mimic MHC I of CMV

Viral protein

Antibody

F$_c$R receptor

B. Reduced MHC I expression on cell surface

NK cell

Inhibitory signal

Mimic MHC I receptor of CMV

Infected cell showing reduced MHC I

Activation signal + Inhibitory signal = no killing

Cytomegalovirus

Activation signal
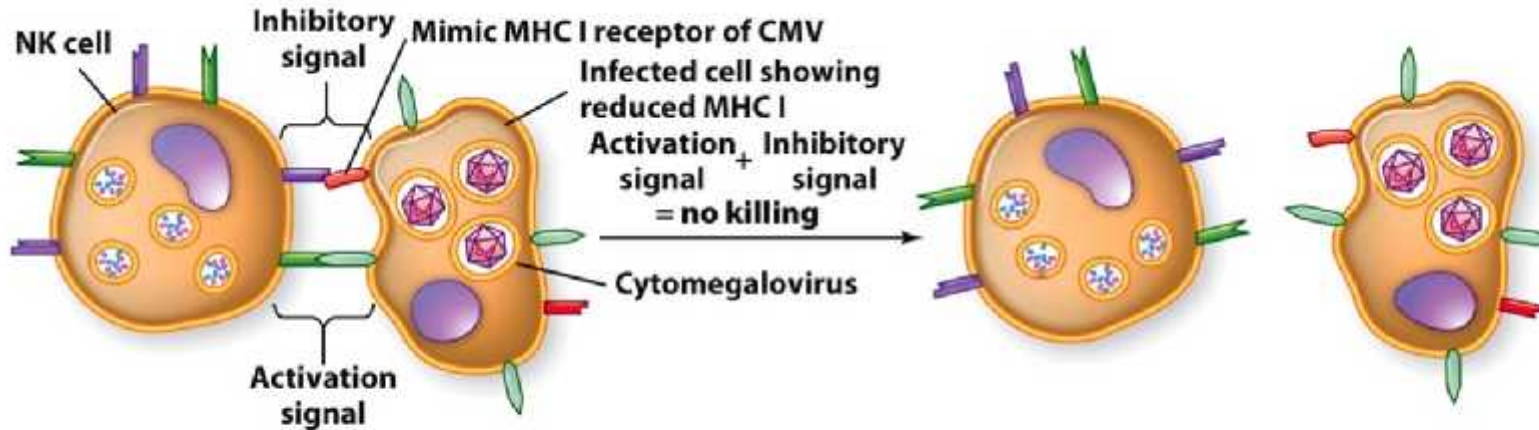
C. Cytomegalovirus mimicry of MHC I

Antibody binds to viral proteins.

The F_CRs of the NK cell bind to the antibodies.

Virus-infected cell

Viral protein expressed on cell

Antibody

F_CR + Bound antibody = killing

NK cell releases perforin and granzyme.

Apoptotic bodies will be phagocytosed and destroyed.

Apoptosis

D. Antibody-dependent cell-mediated cytotoxicity (ADCC)

CD94

Mimic MHC I of CMV

MHC I receptor

Viral protein

Activating ligand

Antibody

Activating receptor

F_CR receptor

34
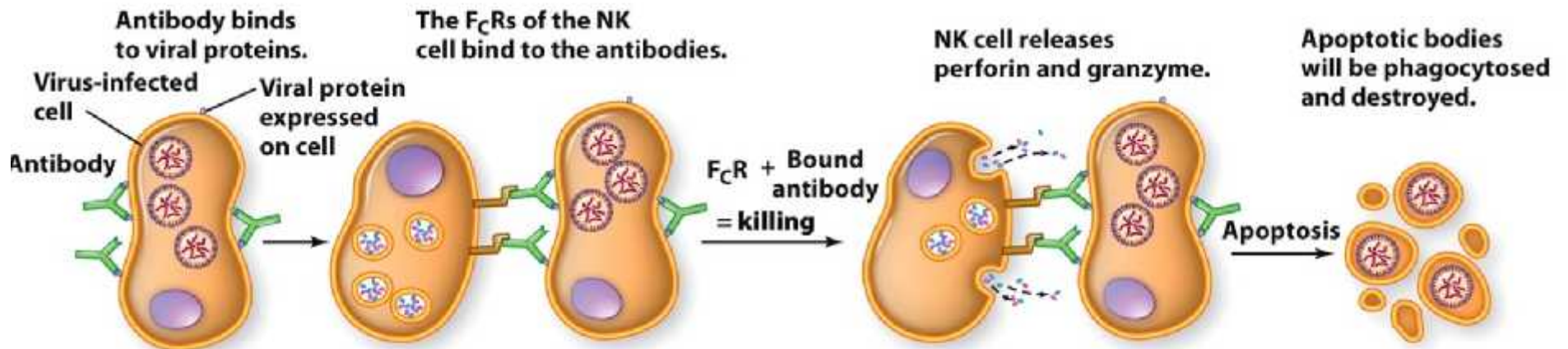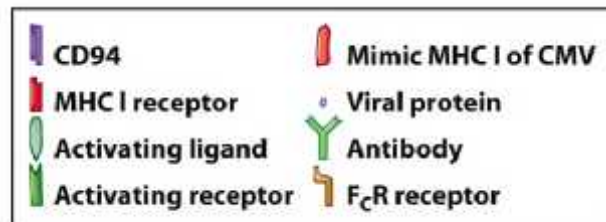
Medical Microbiology

Chapter Three B: Adaptive Immunity
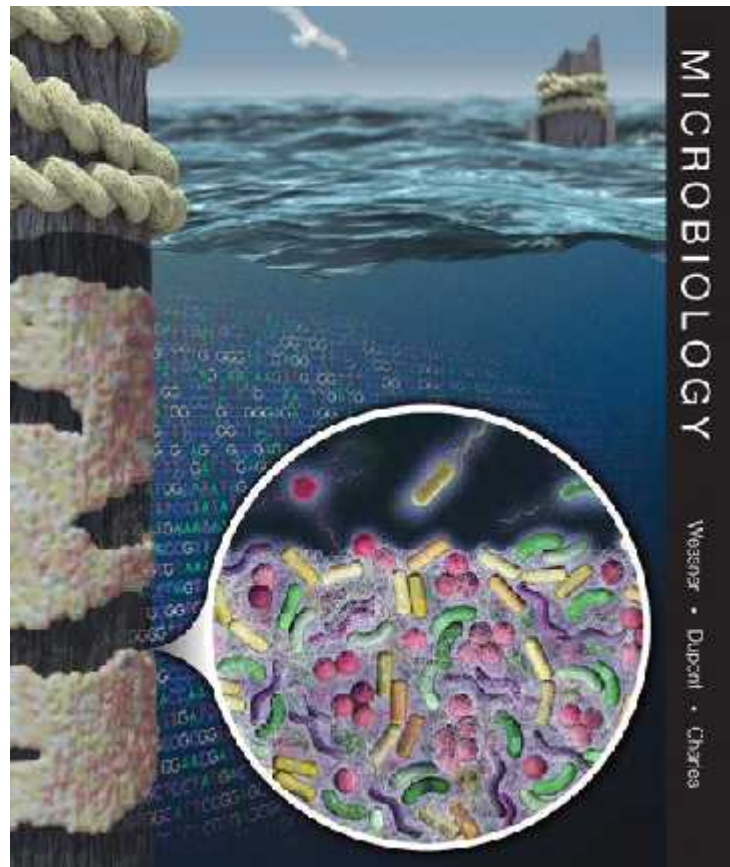
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- What if innate immunity isn't enough to clear an infection?
  - Adaptive immunity can assist.
    - Adaptive immunity is found in jawed or higher vertebrates.
    - The system has a high degree of specificity for individual foreign molecules of pathogens.
    - The system also has the ability to "remember" previous exposures, providing long-term protection against reexposure to the same pathogen(s).

Features of adaptive immunity:

- What is the advantage of adaptive immunity?
  - Immune receptors and antigen
    - Immune receptors bind to antigen.
      - T cells possess the T-cell receptors (TCRs).
      - B cells possess immunoglobulin molecules.
        » B-cell receptors (BCRs) when on the surface of a B cell
        » Antibody is the secreted form of the BCR.

T-cell receptors (TCRs)   B-cell receptors (BCRs)

Antibody

T cells   B cells

Features of adaptive immunity:

B-cell receptor (BCR)

Antibody

B cell
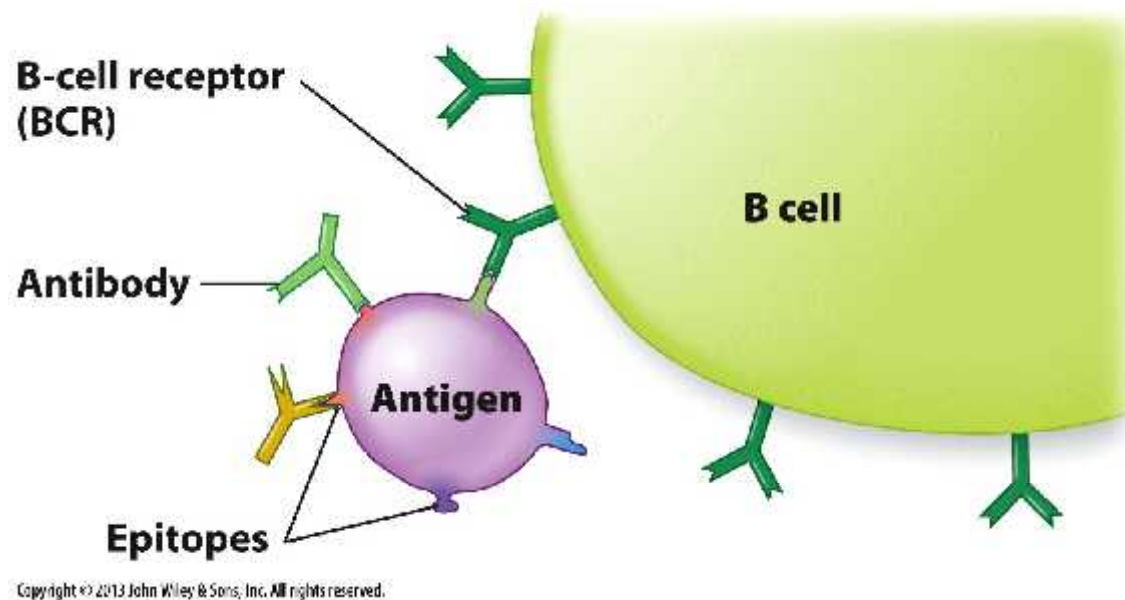
Antigen

Epitopes

- Immune receptors and antigen
  - Only immune receptors can bind antigen.
    - The smallest part of an antigen that can be recognized is an epitope.
    - Each antigen may have multiple different epitopes, each capable of stimulating a response.

Features of adaptive immunity:

- Lymphocytes and lymphoid tissues
  - B and T cells originate in the bone marrow.
    - T cells migrate in a still immature stage to the thymus for further development.
    - Bone marrow and thymus are generative lymphoid organs.
  - During development, gene rearrangements produce a very large number of unique TCRs and BCRs.
    - This increases the chances of a reaction against pathogens.
    - NOTE: The receptors are formed BEFORE exposure to pathogens!

Red blood cells

Lymphocyte

LM

© Biophoto Associates/Photo Researchers, Inc.

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Features of adaptive immunity:

- Lymphocytes and lymphoid tissues
  - Once mature, lymphocytes are expelled into the peripheral blood stream as mature, naïve lymphocytes.
  - These cells migrate through lymphoid tissues distributed around the body, ready to respond to threats.
  - Exposure to a new infectious agent produces a primary immune response.
    - This can take 7–14 days to peak, producing memory lymphocytes as a result and clearing the pathogen.
  - Subsequent exposure results in a memory or anamnestic response (secondary immune response).
    - This response is faster and more potent than the primary.

Memory (secondary) response

Primary response

Immune activity

5   14

Days following exposure to antigen
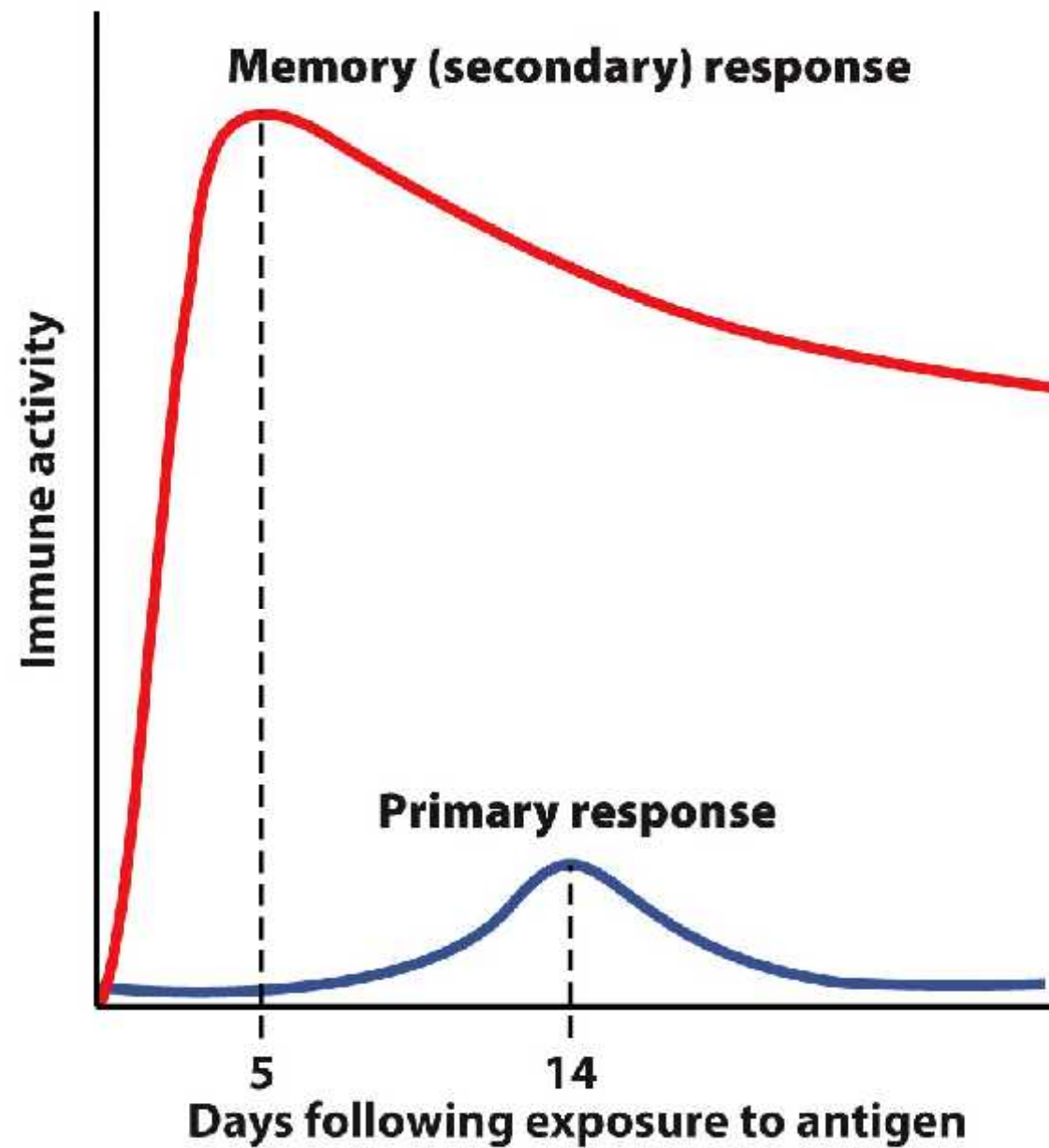
T cells:

- *What are the central cells in adaptive immunity?*
  - Initiation of adaptive immunity is complex.
  - Multiple cell types are involved.
  - T cells are involved with the cell-mediated side of adaptive immunity.
  - Activation of T cells requires
    - Antigen presentation
    - Cell signaling
    - Production of stimulatory molecules
  - Activated T cells expand and differentiate into either effector cells or memory cells.

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T cells:

43
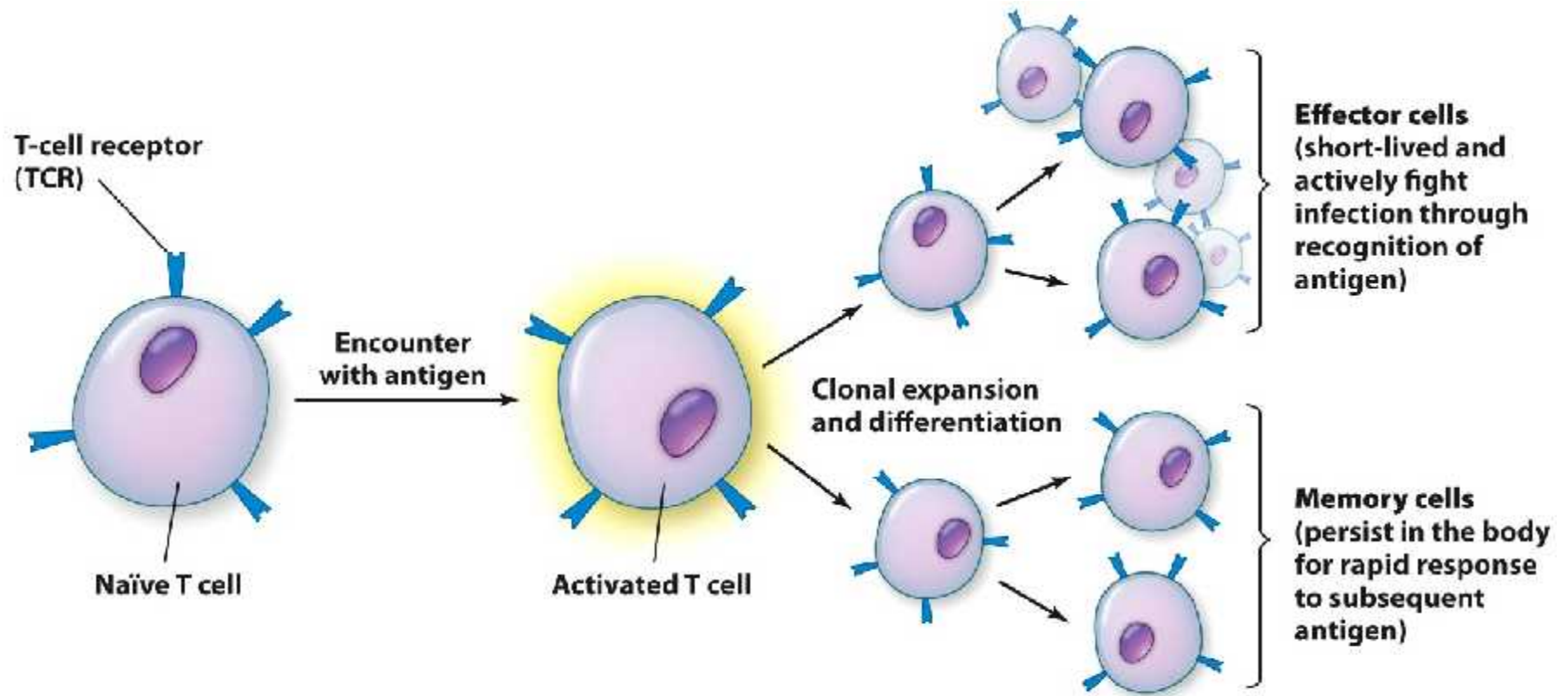
T cells:

- T-cell activation requires binding of the TCR with the specific peptide presented on major histocompatibility (MHC) molecules of APCs.

Naïve T cell

Plasma membrane

T-cell receptor (TCR)

MHC

Peptide antigen

Antigen peptide generated by breakdown of foreign protein

MHC + → MHC:peptide complex

Antigen-presenting cell
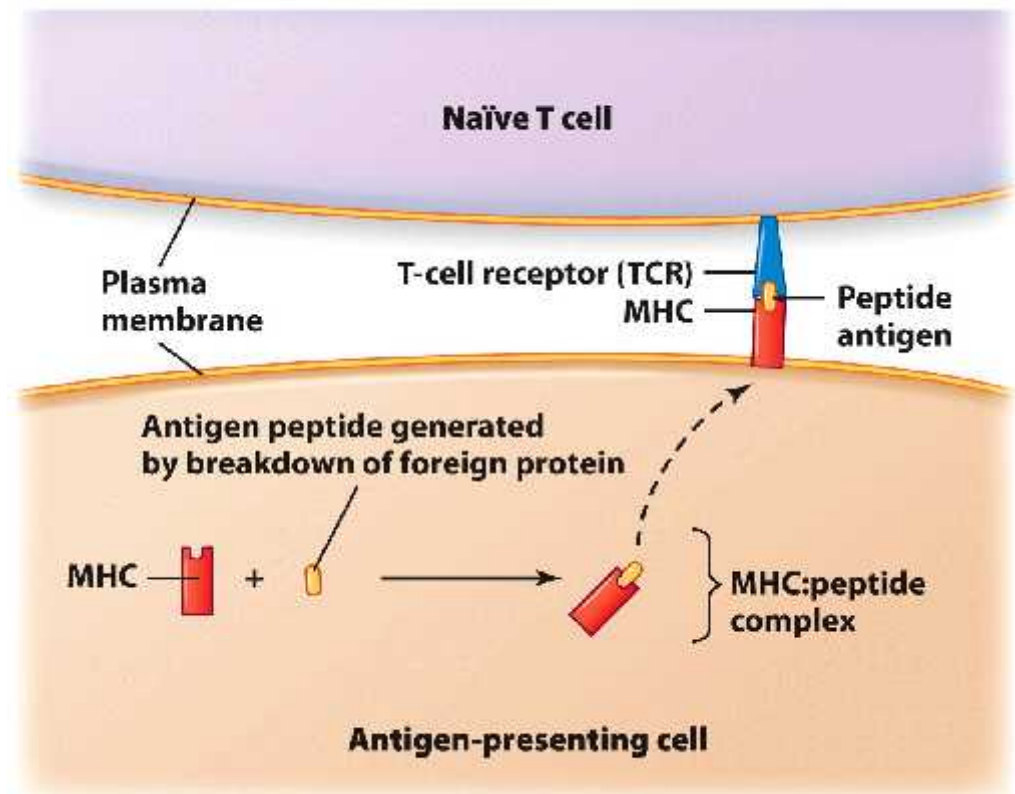
T cells:

- Memory cells and the memory response
  - Establishing memory of previous exposure is a hallmark of adaptive immunity.
  - Memory cells differentiate during initial adaptive immune responses.
    - Long-lived
    - Produce a faster and more vigorous response when the same antigen is encountered again
    - The speed of the 2nd (or 3rd, or 4th) response can even prevent a repeat infection from occurring.

T cells:

- Effector T cells and their functions
  - Effector cells = Action cells
    - Short-lived
    - Armed with direct immune functions
  - In T cells, effector form depends on co-receptors.
    - CD4$^+$ T cells = Secretion of large amounts of cytokine to enhance (help) and direct actions of other immune cells
    - CD8$^+$ T cells = Cytotoxic killing of infected cells by release of granzymes/perforin near contacted target cell, initiating apoptosis
      - Similar to natural killer (NK cells)

① A naïve T cell is activated by an APC.

Naïve T cell

APC

② Clonal expansion occurs producing a population of effector and memory T cells.

⑥ Activation and clonal expansion of the memory T cell into effector and memory T cells occurs.

Memory T cell

Effector T cell

③ Effector T cells migrate to the site of infection and bind to infected cells.

⑤ A late encounter occurs between a memory T cell and an APC presenting the same antigen.

Infected cell

Effector T cell

④ Binding causes activation of the effector function, such as cytotoxic killing of the infected cell.

Memory T cell

Antigen-presenting cell

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Antigen processing:

- Recognition and killing of target cell by an effector cytotoxic T cell…

Cytotoxic T cell (T$_C$ cell)

Granules containing granzymes and perforin

CD8 co-receptor

TCR

Target cell

MHC I:peptide complex: peptide derived from infecting microbe

Intracellular microbe

1) The T$_C$ cell binds to the infected target cell causing degranulation.

2) The target cell undergoes apoptosis.

3) The cell fragments and microbes will be degraded by phagocytes.
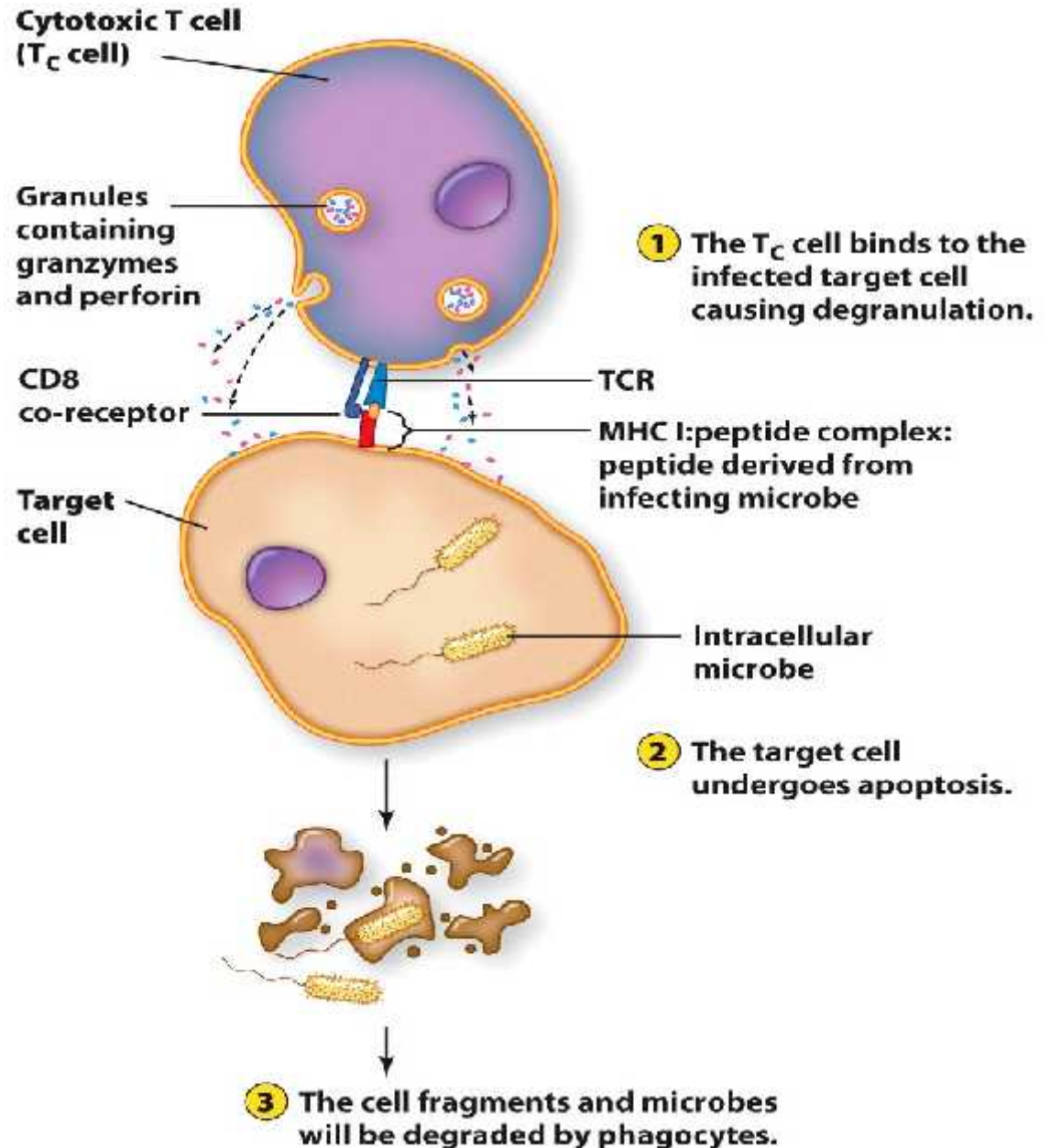
- *What cells can launch adaptive immune responses?*
  - Not all cells are capable of proper antigen presentation to stimulate T cell activation…some are "professional" APCs.

TABLE 20.2 **Activities of antigen-presenting cells**
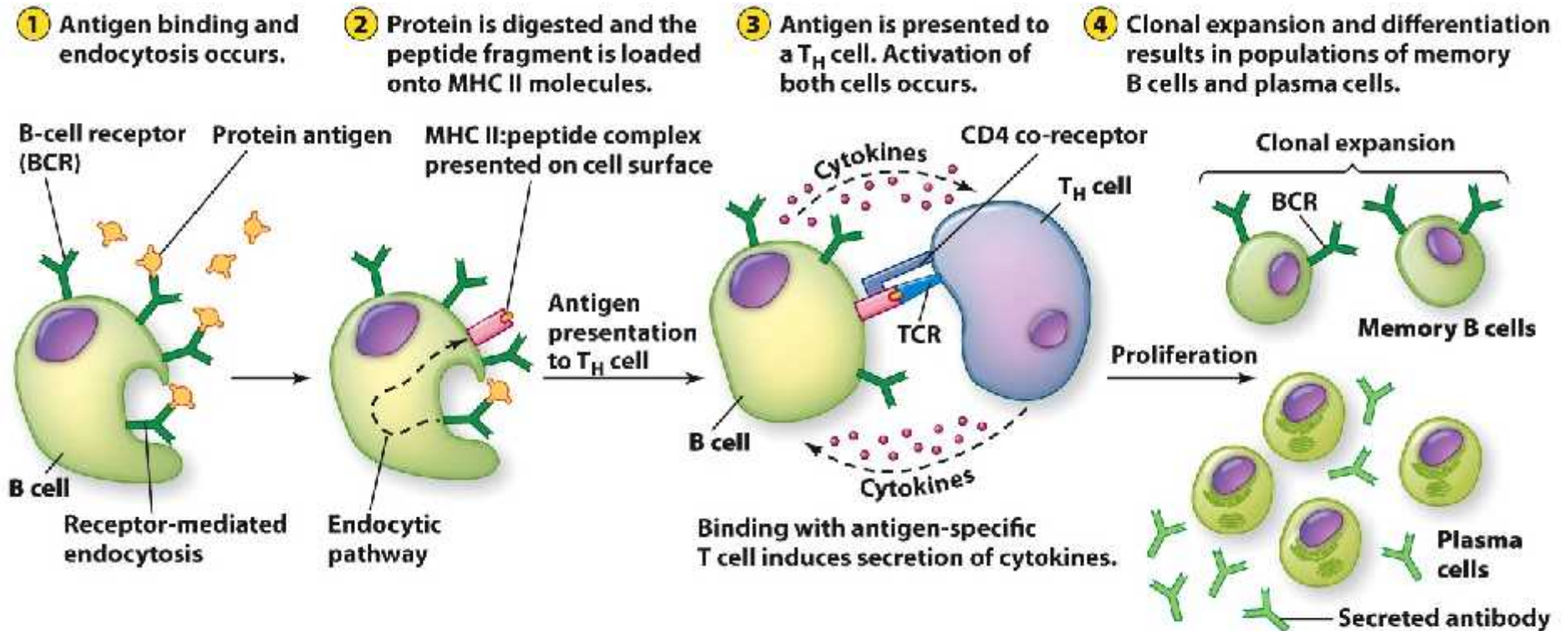
Antigen-presenting cell	Activated cell types	MHC restriction	Outcome
Dendritic cell	Naïve CD4 T cells	MHC II	Primary response; generation of memory and effector CD4 T cells
	Naïve CD8 T cells	MHC I	Primary response; generation of memory and effector CD8 T cells
	Memory CD4 T cells	MHC II	Memory response; generation of effector CD4 T cells
	Memory CD8 T cells	MHC I	Memory response; generation of effector CD8 T cells
	Effector CD4 T cells	MHC II	T_H cytokine secretion
	Effector CD8 T cells	MHC I	T_C cytotoxic killing
Macrophage	Naïve CD8 T cells[a]	MHC I	Primary response; generation of memory and effector CD8 T cells
	Effector CD4 T cells	MHC II	T_H cytokine secretion
	Effector CD8 T cells	MHC I	T_C cytotoxic killing
	Memory CD4 T cells	MHC II	Memory response; generation of effector CD4 T cells
	Memory CD8 T cells	MHC I	Memory response; generation of effector CD8 T cells
B cell	Effector CD4 T cells	MHC II	T_H2 cytokine secretion and B-cell proliferation

[a]Only in certain instances.

- B cells (wait, WHAT?)
  - Critical cells for adaptive immunity
    - Produce antibody
    - Present MHC II:peptide to CD4$^+$ T cells
  - B-cell receptors (BCRs) trap foreign antigen.
    - Antigen is ingested and broken into fragments.
    - Fragments are loaded into MHC class II molecules.
    - Presentation to CD4$^+$ T cells, inducing them to secrete cytokines.
    - The cytokines help the B cell to differentiate into an antibody-secreting plasma cell and/or a memory B cell.
  - Most B cells need the help of T-cell cytokines to become fully activated—an they can't get this help without presenting antigens.

Antigen-presenting cells:

① Antigen binding and endocytosis occurs.

② Protein is digested and the peptide fragment is loaded onto MHC II molecules.

③ Antigen is presented to a T$_H$ cell. Activation of both cells occurs.

④ Clonal expansion and differentiation results in populations of memory B cells and plasma cells.

B-cell receptor (BCR)
Protein antigen
MHC II:peptide complex presented on cell surface
Cytokines
CD4 co-receptor
T$_H$ cell
Clonal expansion
BCR

B cell
Antigen presentation to T$_H$ cell
TCR
Memory B cells

Receptor-mediated endocytosis
Endocytic pathway
B cell
Proliferation

Binding with antigen-specific T cell induces secretion of cytokines.
Cytokines
Plasma cells
Secreted antibody

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Humoral and cell-mediated immune responses:

- Are there different responses to extracellular and intracellular pathogens?
  - Cytokine secretion by T_H cells is required for proper activities of B cells and T_C cells.
    - But antibodies from B cells wouldn't directly help kill a virally infected cell.
    - And T_C cells wouldn't directly kill bacterial cells.
  - Because of the different immunity needs, there are two broad types of responses.
    - Humoral immunity: Good against extracellular pathogens
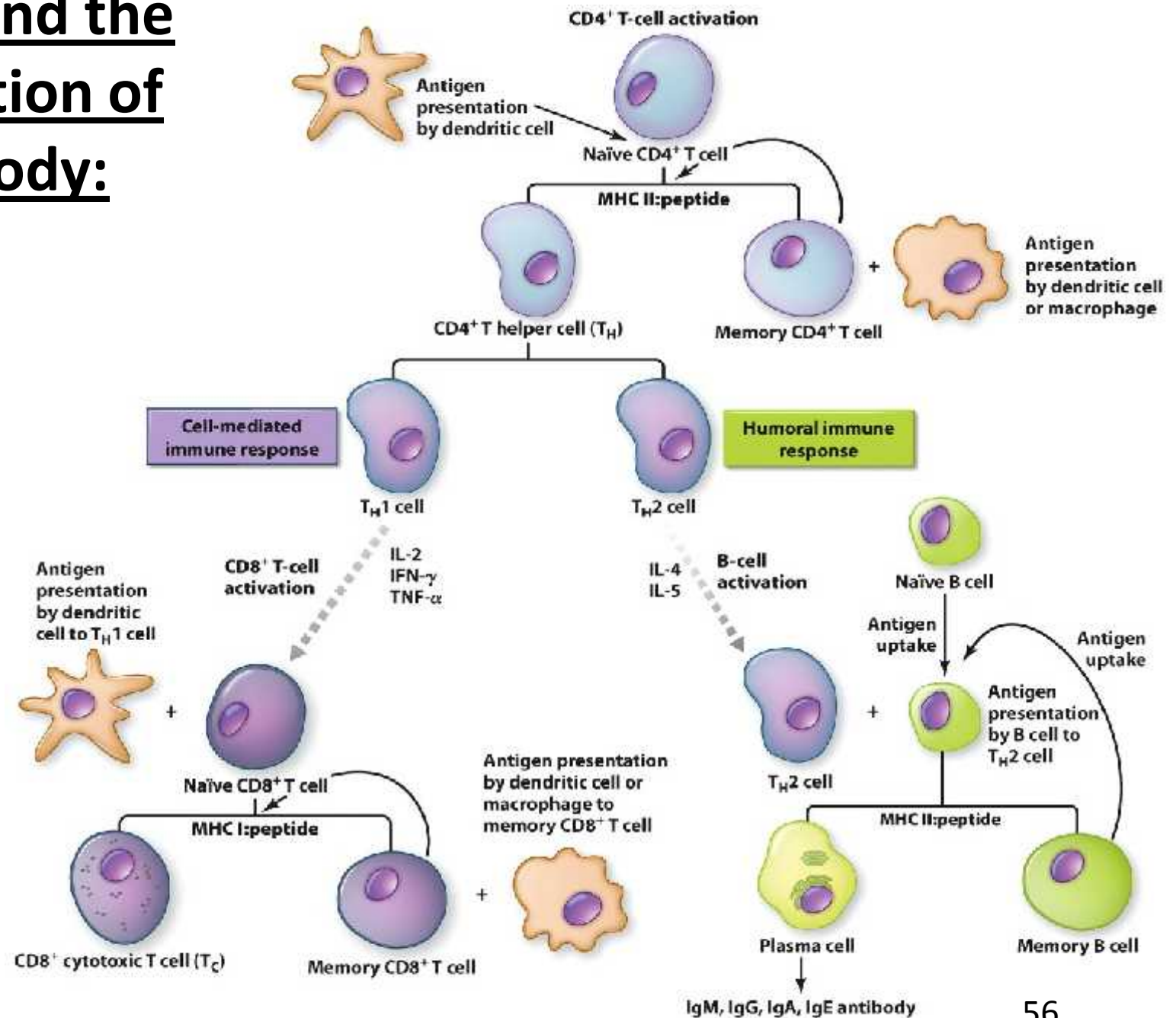   - Cell-mediated immunity: Good against intracellular pathogens

53
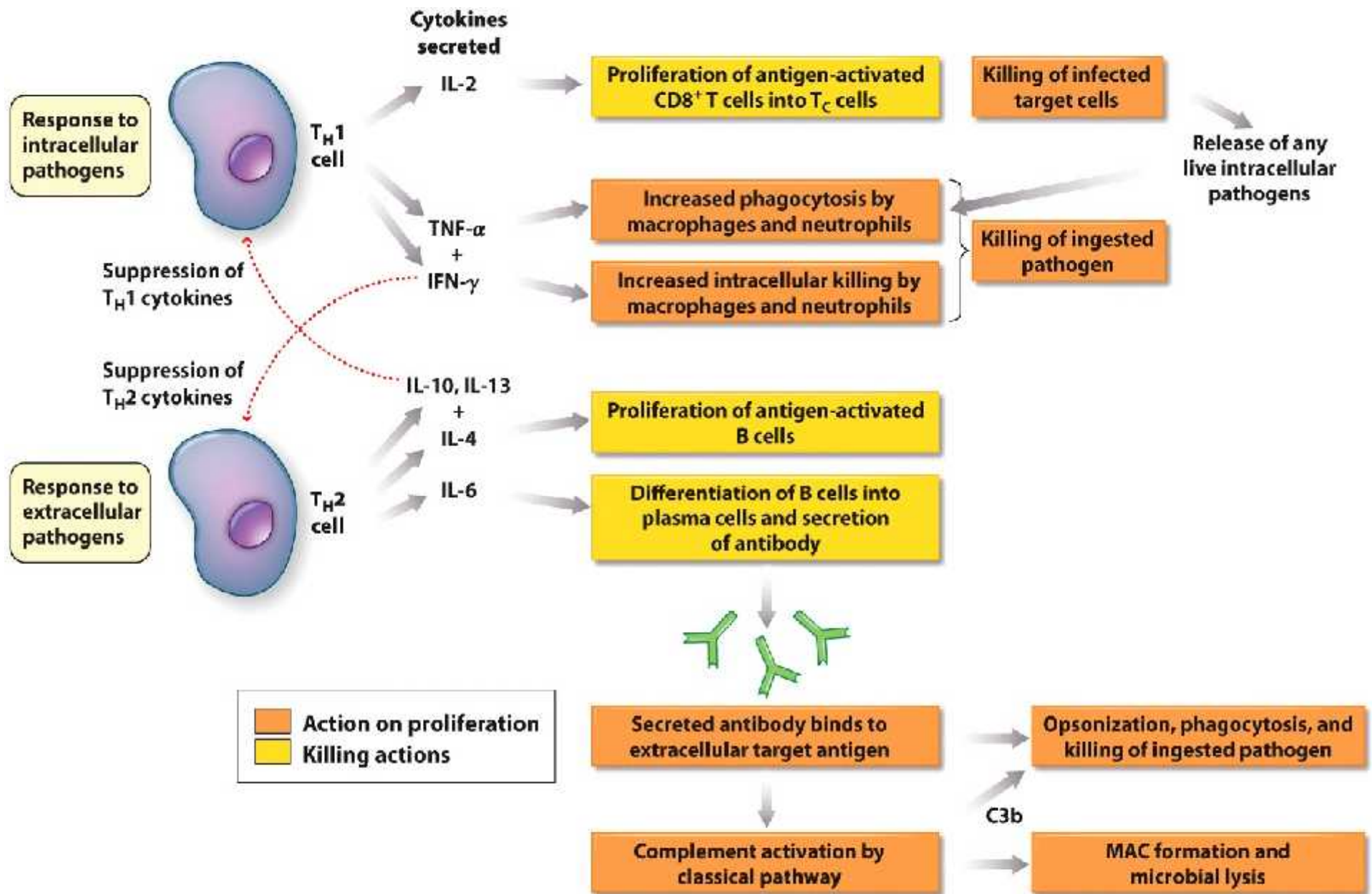
Humoral and cell-mediated immune responses:

- Immune-mediated damage
  - Sometimes, immune responses can cause damage to self structures if they get too strongly stimulated.
    - Meningitis: Excessive pressure on nerves, paralysis and death
    - Gonorrhea: Sterility
    - Superantigens: Result in excessive levels of cytokines to be released from helper T cells
  - Cross reactivity can also be a problem.
    - Antibodies against bacterial structures bind to heart valve proteins, causing damage.
  - Virus infections resulting in immune-based destruction of large numbers of self cells (e.g., hepatitis B)
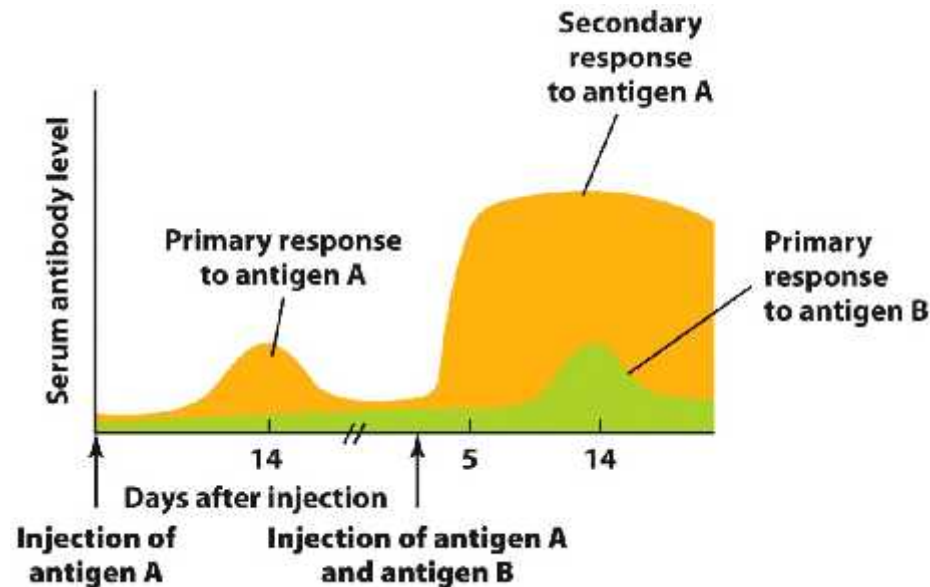
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B cells and the production of antibody:

- *How are antibodies produced?*
  - Recall that our best understanding of how B cells produce antibodies is in response to protein antigens from foreign sources.
    - These responses usually require cytokine help from helper T cells.
      - The B cells must first bind the foreign antigen through the BCR, then ingest it and process it.
      - Presentation of the antigenic peptides to the TCR on the helper T cell solicits the cytokine help.
    - The B cell multiplies, differentiating into antibody-secreting plasma cells and memory cells.

B cells and the production of antibody:

CD4⁺ T-cell activation

Antigen presentation by dendritic cell

Naïve CD4⁺ T cell

MHC II:peptide

CD4⁺ T helper cell (T_H)

Memory CD4⁺ T cell

Antigen presentation by dendritic cell or macrophage

Cell-mediated immune response

Humoral immune response

T_H1 cell

T_H2 cell

Antigen presentation by dendritic cell to T_H1 cell

CD8⁺ T-cell activation

IL-2
IFN-γ
TNF-α

IL-4
IL-5

B-cell activation

Naïve B cell

Antigen uptake

Antigen uptake

Antigen presentation by B cell to T_H2 cell

Naïve CD8⁺ T cell

MHC I:peptide

Antigen presentation by dendritic cell or macrophage to memory CD8⁺ T cell

T_H2 cell

MHC II:peptide

CD8⁺ cytotoxic T cell (T_C)

Memory CD8⁺ T cell

Plasma cell

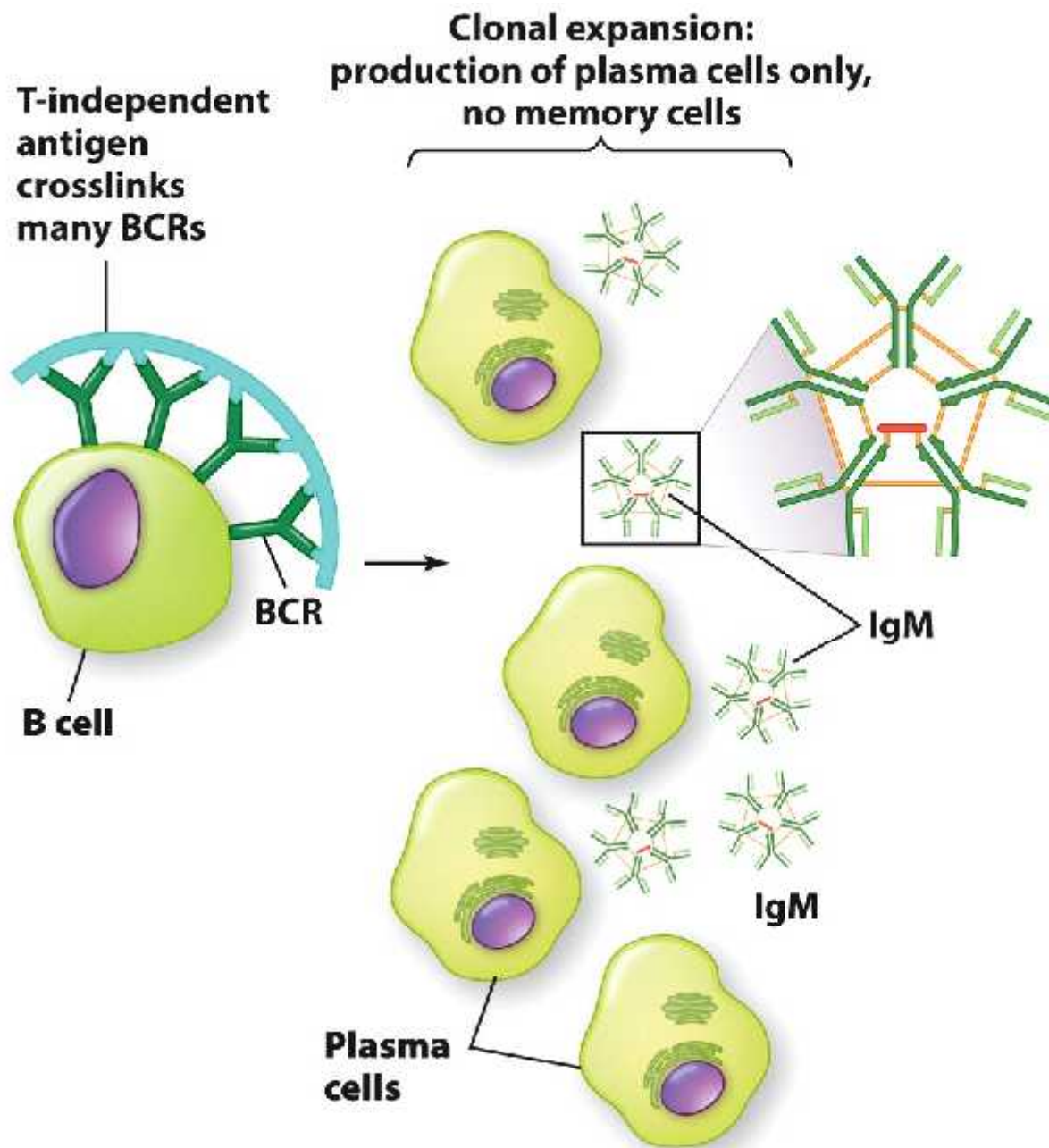Memory B cell

IgM, IgG, IgA, IgE antibody
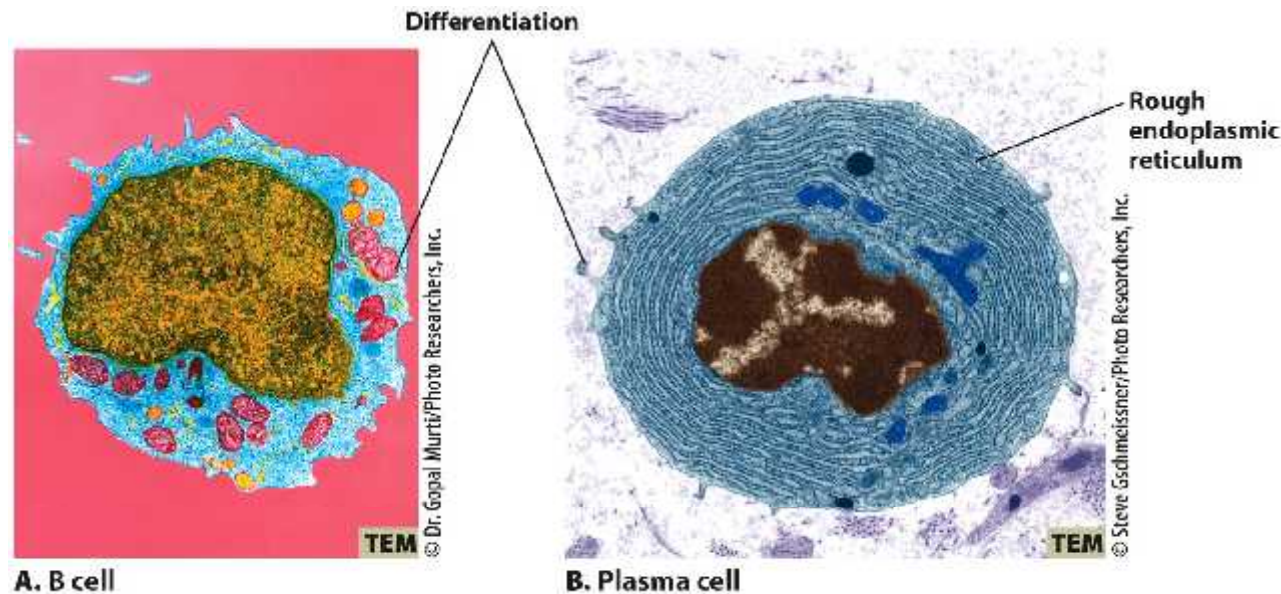
56

B cells and the production of antibody:

- B-cell responses to protein antigens
  - Primary responses typically produce a modest amount of antibody after 7–14 days.
  - Secondary (memory) responses shift to much faster and larger-scale antibody production.
    - But the ability to respond to different antigens is still retained.

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T-independent antigen crosslinks many BCRs

Clonal expansion: production of plasma cells only, no memory cells

BCR

B cell

IgM

IgM

Plasma cells

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B cells and the production of antibody:

Differentiation

A. B cell

B. Plasma cell

Rough endoplasmic reticulum

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© Steve Gschmeissner/Photo Researchers, Inc.

TEM

TEM

- • Antibody production by plasma cells
  - – Plasma cells can secrete 2,100+ antibodies per SECOND.
  - – Terminally differentiated (cannot perform cell division)
    - • All produced from proliferation/differentiation of activated naïve or memory B cells.
    - • Quickly undergo apoptosis (in about 2 days), but the antibodies they secrete may last for weeks in the blood.
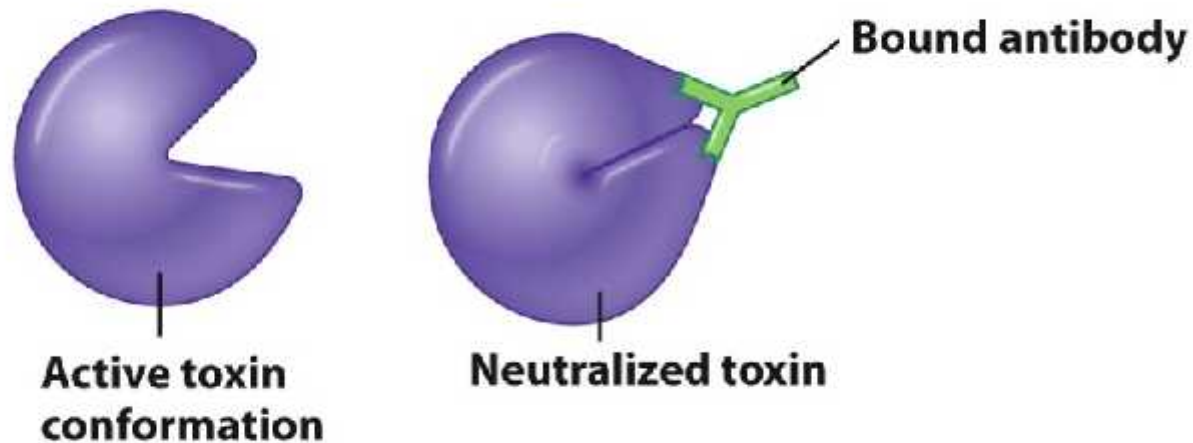
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B cells and the production of antibody:

- Protection by antibodies
  - So now we know how antibody is produced and what it looks like. What does it DO to protect us?
    - Blocks binding of pathogens/toxins on host cells
    - Fixes complement to bacterial structures, leading to lysis
    - Opsonization (increases phagocytosis)
    - Agglutination (clumping of antigen, increasing phagocytosis)
    - Activates eosinophils, basophils, and mast cells by providing an exposed FC region for the cells to bind to
    - Antibody-dependent cell-mediated cytotoxicity (ADCC) killing of infected cells by natural killer (NK) cells.

- Protection by antibodies

Bound antibody   **Host cell**

Virus

Virus receptor
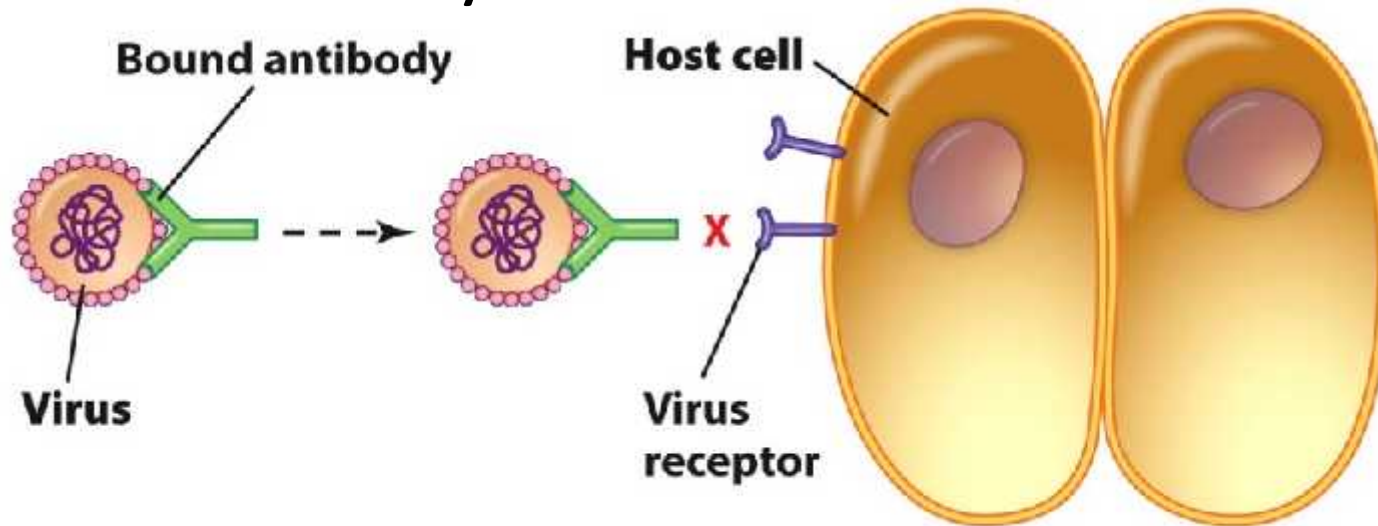
A. Prevention of binding to receptors

Active toxin conformation
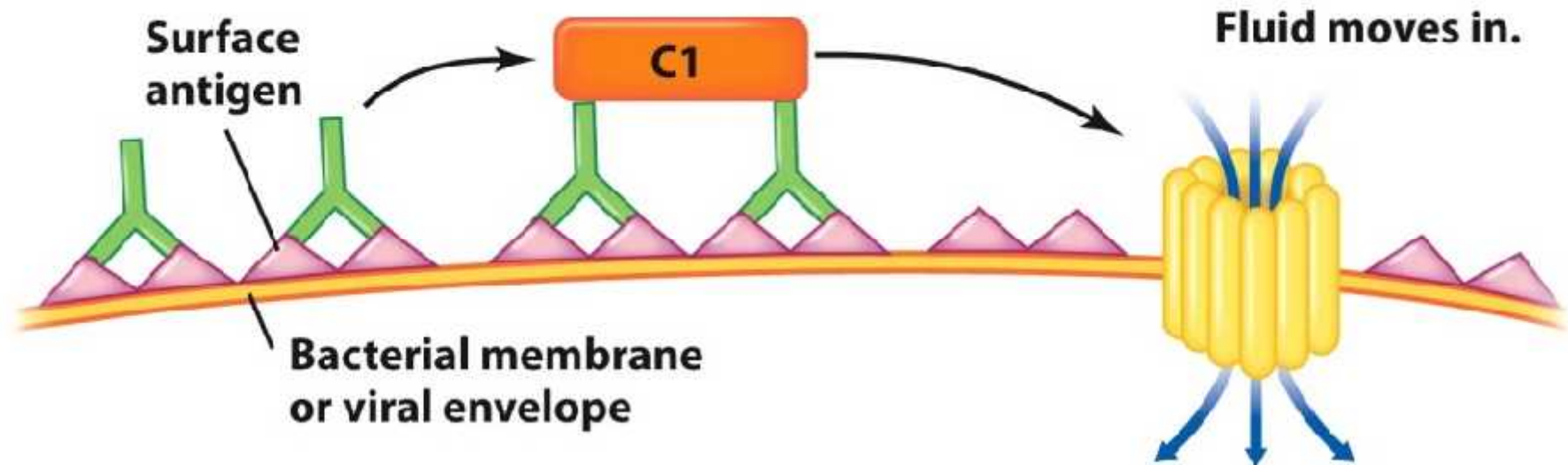
Bound antibody

Neutralized toxin

B. Prevention of conformational changes

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1 Antibody binds to surface of microbe.

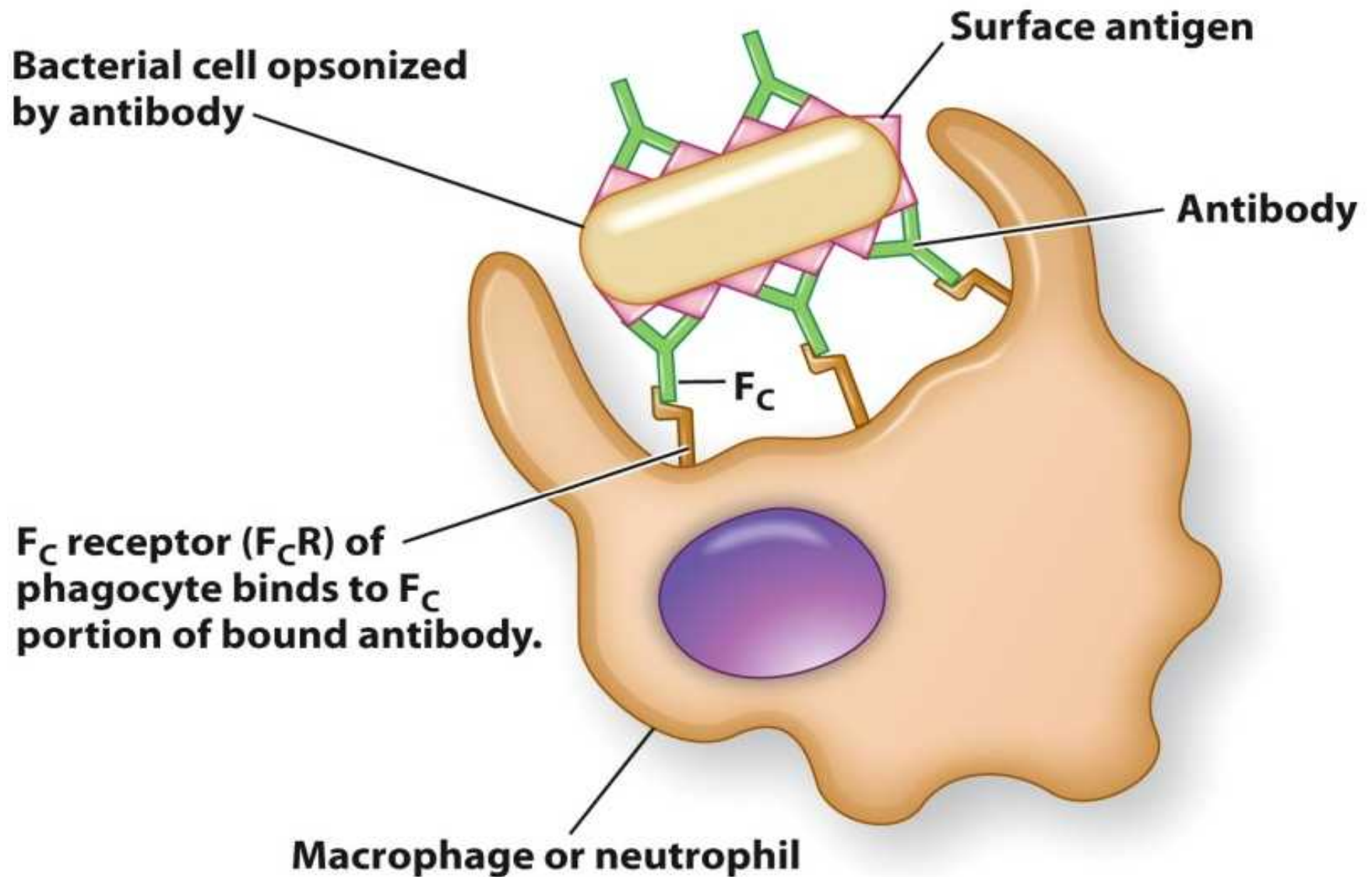**2** Complement C1 binds to antibody F_C portion, initiating the complement cascade.

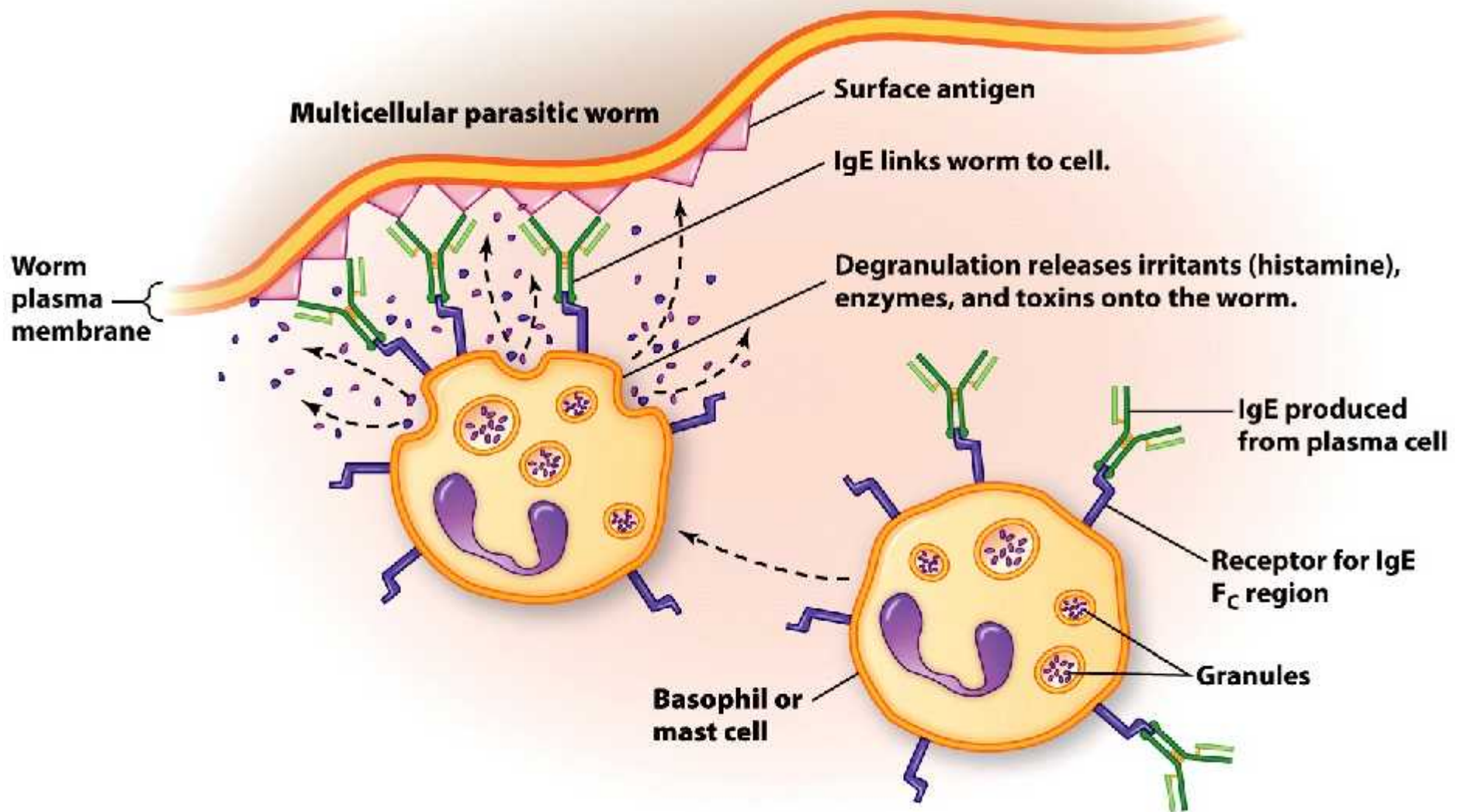**3** MAC forms, resulting in membrane disruption.

Exterior

Surface antigen

C1

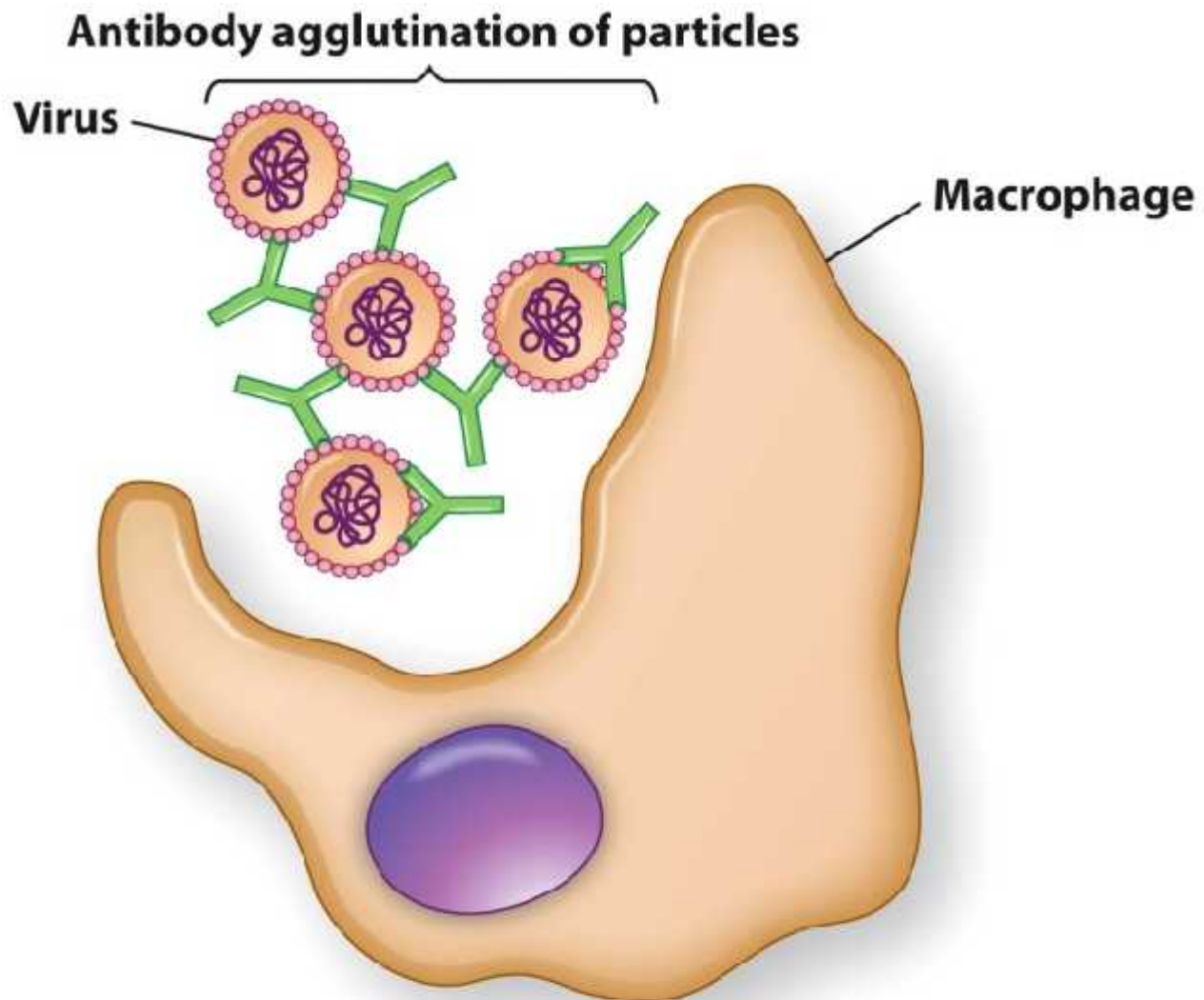Fluid moves in.

Bacterial membrane or viral envelope

Interior

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Bacterial cell opsonized by antibody

Surface antigen

Antibody

F_C

F_C receptor (F_CR) of phagocyte binds to F_C portion of bound antibody.

Macrophage or neutrophil

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Multicellular parasitic worm

Surface antigen

IgE links worm to cell.

Worm plasma membrane

Degranulation releases irritants (histamine), enzymes, and toxins onto the worm.

IgE produced from plasma cell

Receptor for IgE F_C region

Granules

Basophil or mast cell

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Antibody agglutination of particles

Virus

Macrophage

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Conclusion:

- Adaptive immunity is very complex.
  - The protective elements are highly specific but take time to develop.
  - The fundamental difference from innate immunity is the development of memory.
  - Oftentimes, innate and adaptive immunity work together to clear pathogens.
  - Adaptive immunity can cause damage to our structures if it is inappropriately or excessively stimulated.
  - We can use adaptive immunity in medical and research settings as a useful tool.
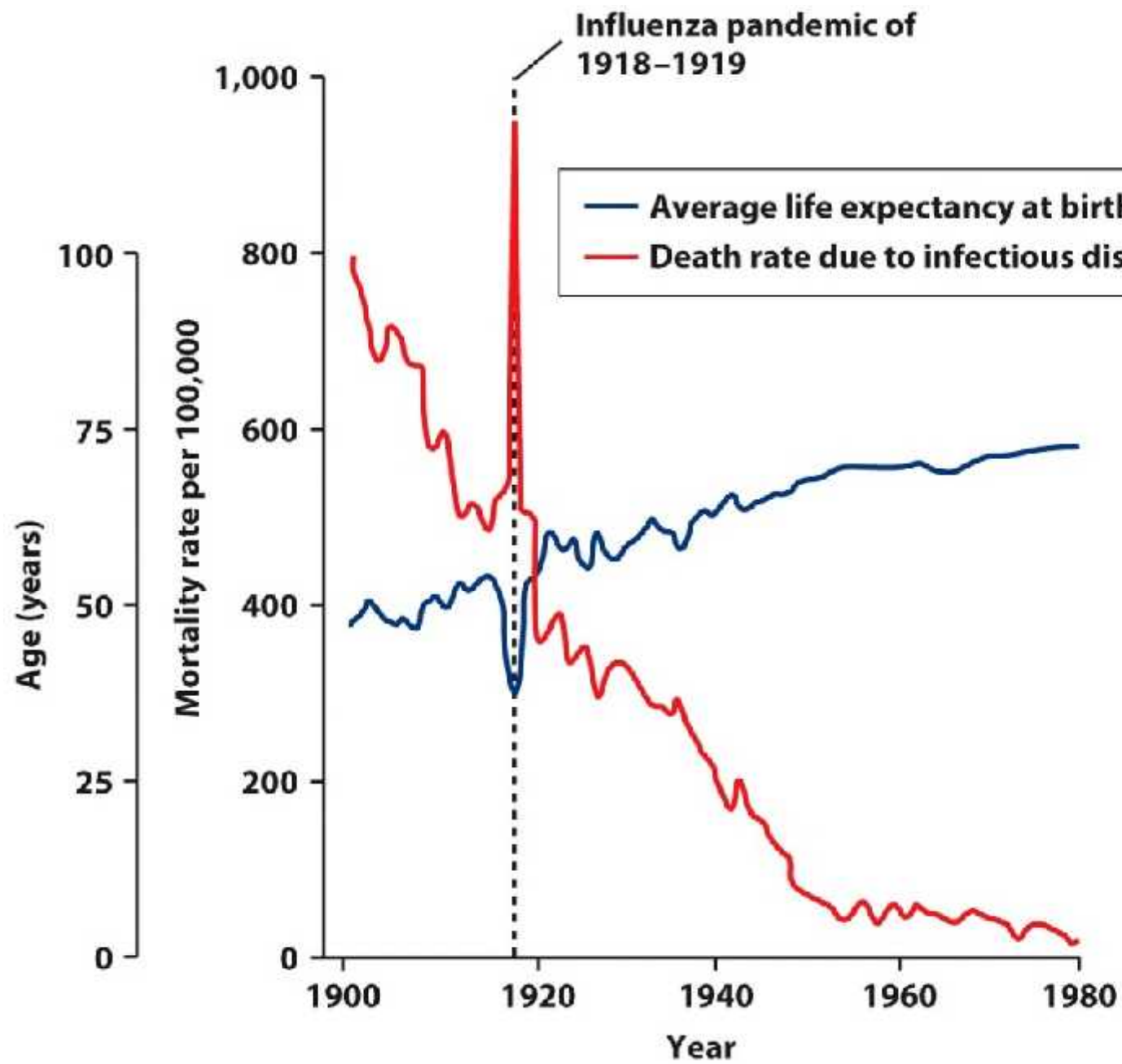
Medical Microbiology

Chapter Four : Introduction to Infectious Diseases
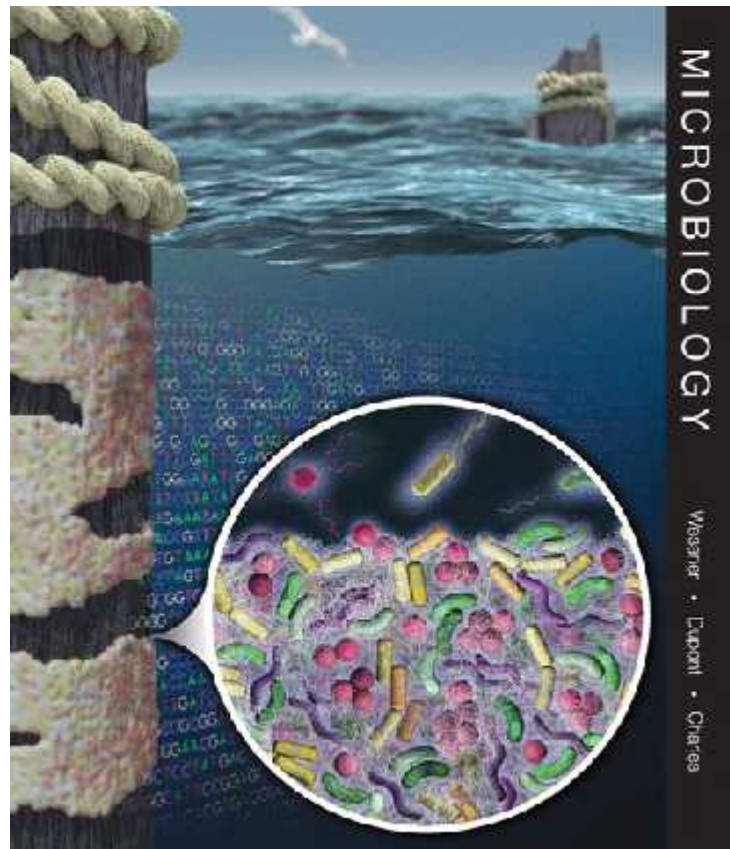

Dr. Sulaiman Alnaimat 2015

Introduction:

- Diseases have long ravaged human populations.
  - Bubonic plague in the 1300s

  - Exploration of the Americas in the 1500–1600s bringing European diseases to indigenous humans

  - Spanish flu in the early 1900s

Influenza pandemic of 1918–1919

Average life expectancy at birth (both sexes)
Death rate due to infectious disease

Age (years)

Mortality rate per 100,000

Year

Courtesy of the Marian Koshland Science Museum of the National Academy of Sciences

3

- Despite advances in vaccinations, antibiotics, sanitation, and medical care, infectious diseases still take a heavy toll on human life.
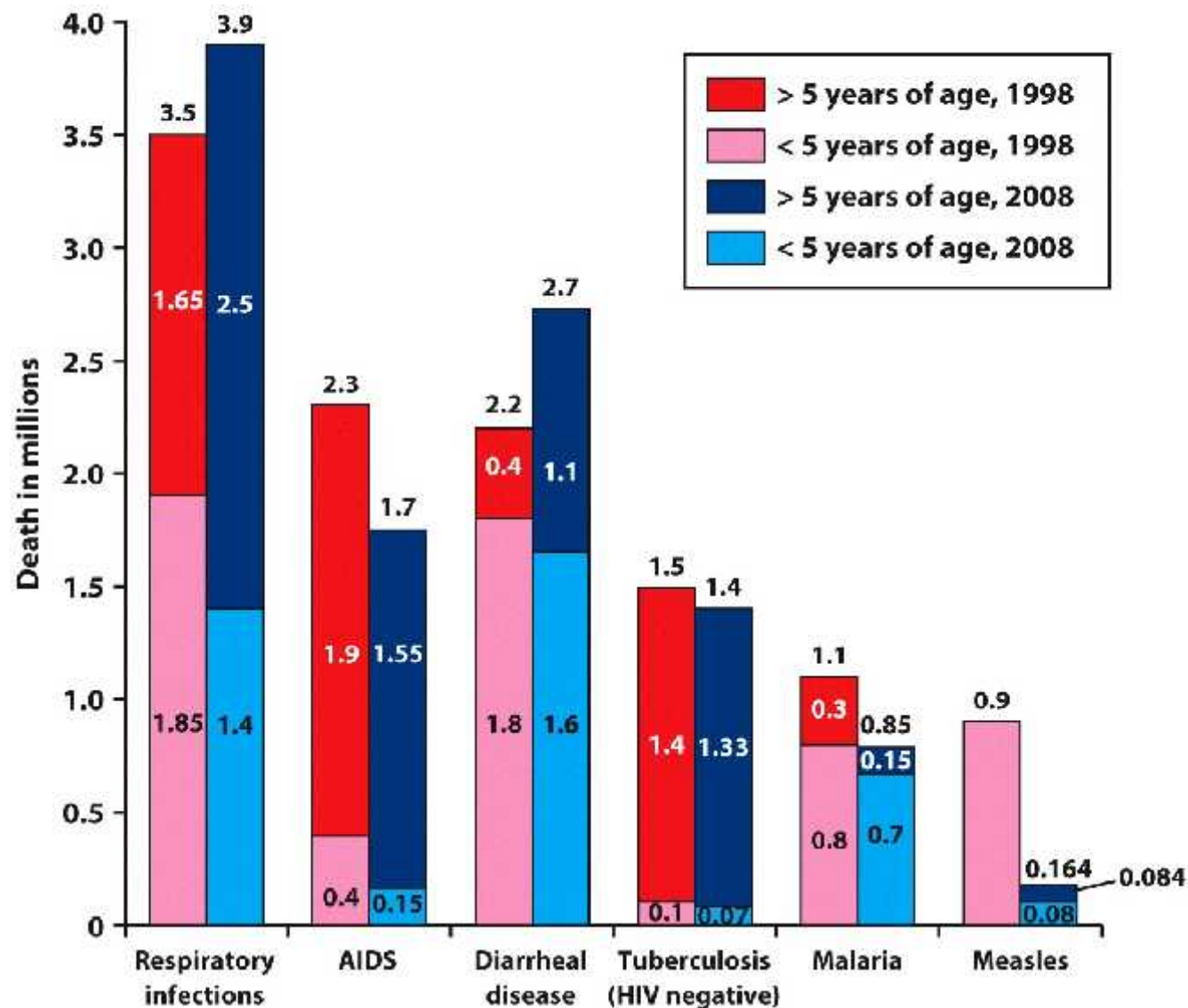
Pathogenic microbes:

- *What is an infectious disease?*
- – Disease = A disturbance in normal functioning of an organism
  - – Infectious disease is caused by a microbe and can be transmitted from host to host.
    - Influenza, HIV, hepatitis B
  - – Zoonotic diseases are infectious diseases of animals that can cause disease when transmitted to humans.
    - Rabies, West Nile fever

Pathogenic microbes:

- **Pathogens** = Microbes frequently associated with disease production
  - Pathogenesis is the mechanism a microbe uses to cause the disease state.
  - Infection refers to the replication of a pathogen in or on its host.
  - Specific signs/symptoms are associated with specific diseases.

TABLE 18.1 Signs and symptoms of specific infectious diseases			
Disease	**Cause**	**Signs**	**Symptoms**
Acquired immunodeficiency syndrome (AIDS)	Human immunodeficiency virus	Opportunistic infections, decreased $CD4^+$ T cell count	Fatigue, muscle aches
Malaria	Plasmodium falciparum	Fever, anemia	Muscle pain, headaches
Flu	Influenza virus	Fever	Muscle aches, malaise
Salmonellosis	Salmonella Typhimurium	Diarrhea, fever	Abdominal cramps

Pathogenic microbes:

- Not all pathogens have the same ability to cause disease.
  - Primary pathogens tend to produce disease readily in healthy hosts.
  - Opportunistic pathogens generally only cause disease when displaced to an unusual site or when the host has a weakened immune system.

TABLE 18.2 **Examples of primary and opportunistic bacterial pathogens**

Category	Bacterium	Disease
Primary pathogen	*Bacillus anthracis*	Anthrax
	Bordetella pertussis	Whooping cough
	Borellia burgdorferi	Lyme disease
	Corynebacterium diphtheriae (lysogenized strains)	Diphtheria
	Escherichia coli O157:H7	Hemorrhagic colitis, kidney disease
	Helicobacter pylori	Gastritis and ulcers
	Mycobacterium tuberculosis	Tuberculosis
	Rickettsia typhii	Typhus
	Salmonella Typhi[a]	Typhoid fever
	Salmonella Typhimurium[a]	Salmonellosis
	Treponema pallidum	Syphilis
	Vibrio cholerae	Cholera
	Yersinia pestis	Plague

TABLE 18.2 Examples of primary and opportunistic bacterial pathogens

Category	Bacterium	Disease
Opportunistic pathogen	Clostridium botulinum	Botulism
	Clostridium difficile	Pseudomembranous colitis
	Clostridium perfringens	Gas gangrene
	Clostridium tetani	Tetanus
	Enterobacter aerogenes	Urinary, respiratory infections
	Escherichia coli uropathogenic strains	Urinary tract infections
	Haemophilus influenzae	Meningitis, pneumonia
	Klebsiella pneumoniae	Pneumonia
	Legionella pneumocystis	Pneumonia, septicemia
	Listeria monocytogenes	Meningitis, septicemia
	Mycoplasma pneumonia	Atypical pneumonia
	Neisseria meningitidis	Meningitis
	Pseudomonas aeruginosa	Pneumonia, skin infections
	Serratia marcescens	Urinary tract infections, endocarditis
	Staphylococcus aureus (various strains)	Skin infections, toxic shock syndrome, pneumonia, endocarditis, vascular infections
	Streptococcus agalactiae	Newborn meningitis
	Streptococcus mutans	Tooth decay, endocarditis
	Streptococcus pneumoniae	Meningitis, pneumonia
	Streptococcus pyogenes	Pharyngitis, scarlet fever, rheumatic fever, necrotizing fasciitis, toxic shock syndrome

Pathogenic microbes:

- Virulence = Measure of the severity of disease a pathogen can induce
  - Pathogens can weaken over time or show different virulence levels due to genetic differences.
    - Attenuated strains show decreased virulence.
      - May be useful for vaccine development
   - Avirulent strains can no longer cause disease.

Observation: The *Yersinia pestis* adenylate kinase (AK) enzyme is involved in the biosynthesis of adenosine.

FM

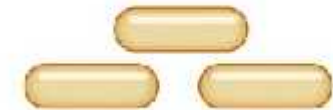$$ATP + AMP \xrightleftharpoons{AK} ADP + ADP$$
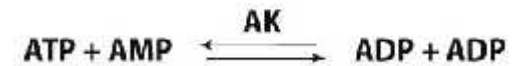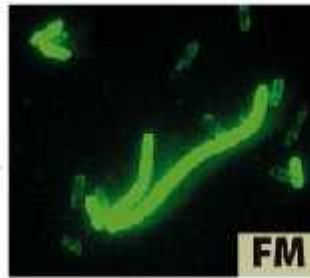
Hypothesis: Thermosensitive mutants of AK will lead to decreased growth at the non-permissive temperature 37°C.

Experiment: Mutate codon 87 of AK gene, changing the amino acid from proline to serine. Compare pathogenesis of wild-type and mutant strains.
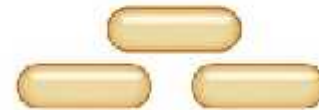
Y. pestis expressing wild-type AK containing proline

Infects mice

Y. pestis expressing mutant AK (AK_{P87S}) containing serine

Infects mice

Results: AK_{P87S} mutant exhibited greatly decreased pathogenesis.

Conclusion: Adenylate kinase contributes to the pathogenesis of *Y. pestis*.

Y. pestis strain	CFU injected (iv)	% Lethality
Wild type	0	0
	3.6	20
	36	100
AK_{P87S}	0	0
	150	0
	15,000	0

10

Pathogenic microbes:

- Carrier = Individual infected with a pathogenic microbe who never exhibits overt signs or symptoms of the disease (asymptomatic).
  - The asymptomatic host may still be able to transmit the microbe to others.
  - A famous carrier case was Mary Mallon (aka Typhoid Mary).

Mary Mallon (September 23, 1869 – November 11, 1938), better known as Typhoid Mary, was the first person in the United States identified as an asymptomatic carrier of the pathogen associated with typhoid fever. She was presumed to have infected 49 people, three of whom died, over the course of her career as a cook. She was twice forcibly isolated by public health authorities and died after a total of nearly three decades in isolation. **Wikipedia**

Microbial virulence strategies:

- How do microbes cause disease?
- To cause an infection, most pathogens must

  1. Gain entry to the host
  2. Attach to and invade specific cells and/or tissues within the host
  3. Evade host defenses
  4. Obtain nutrients from the host
  5. Exit the host

- Frequently, cellular damage occurs as a result of these processes mediated by pathogenic microbial products.

TABLE 18.3   **Virulence actions of various pathogens**

Virulence action	Viruses	Bacteria
Attachment and invasion	HIV: Viral gp120 protein attaches to CD4 molecule found on certain immune system cells.	*Escherichia coli*: Enteropathogenic and enterohemorrhagic strains produce intimin, an adhesion molecule.
Evade host defenses	Herpes simplex virus: Following acute replication, the viral genome exists as an episome in the host cell nucleus, undergoing limited transcription and translation.	*Neisseria gonorrhoeae*: The organism produces IgA protease, an enzyme that destroys the host IgA antibodies.
Obtain nutrients from the host	Human papillomavirus: Viral E6 and E7 proteins inhibit cellular p53 and Rb tumor suppressor proteins, thereby driving the cell to replicate and produce molecular building blocks needed by the virus.	*Mycobacterium tuberculosis*: Mycobactins, iron-binding proteins, allow the pathogen to acquire iron from the host.
Cause cellular damage	Poliovirus: Replication induces apoptosis of host cells.	*Clostridium botulinum*: The organism produces botulinum toxin, a powerful neurotoxin that results in flaccid paralysis.

Microbial virulence strategies:

- Attachment, invasion, and replication
  - Attachment may occur through specific protein:protein interactions.
    - Viruses often utilize a specific host cell receptor.
  - Occasionally, attachment may occur through more generalized interactions.
    - Rice blast fungus spores adhere to most hydrophobic surfaces, including cells.

B. pertussis colonizing the trachea

Cilia

Bacteria

Nonciliated cells

2 μm

Figure 25.14b Microbiology: An Evolving Science
NIBSC/Science Photo Library

This slide has bee taken from **Slonczewski, Joan, John Watkins Foster, and Kathy M. Gillen.** *Microbiology: an evolving science*. WW Norton, 2011.
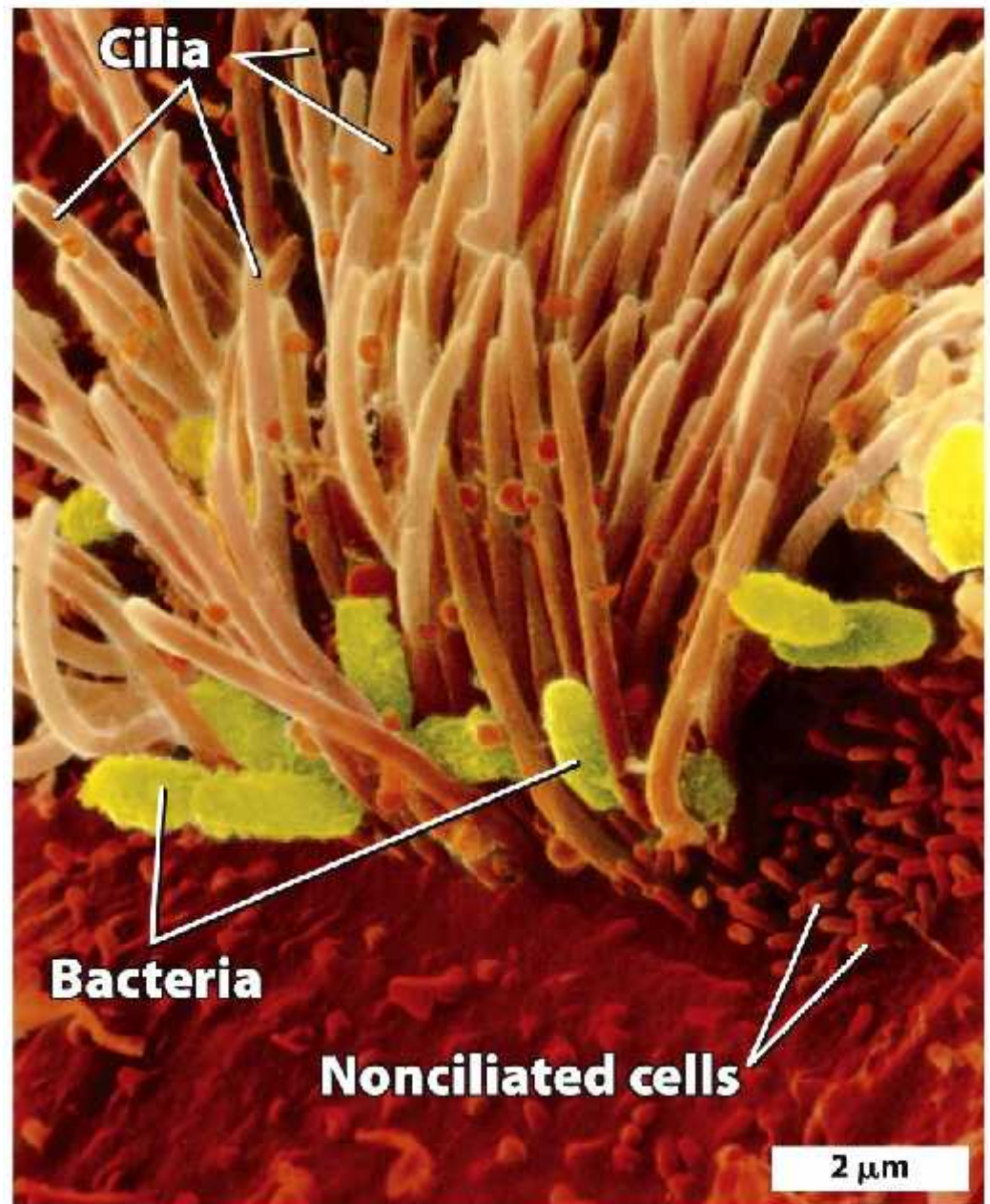
Microbial virulence strategies:

- Attachment, invasion, and replication
  - Host range = The group of organisms that the pathogen can infect
    - Determined by the pathogen's ability to attach, invade, and replicate within a host
    - An example of the spread of a pathogen into a new host (expanding its range) via mutation lies in the spread of FPLV (in cats) to become CPV (in dogs).

Microbial virulence strategies:

- Evading host defenses
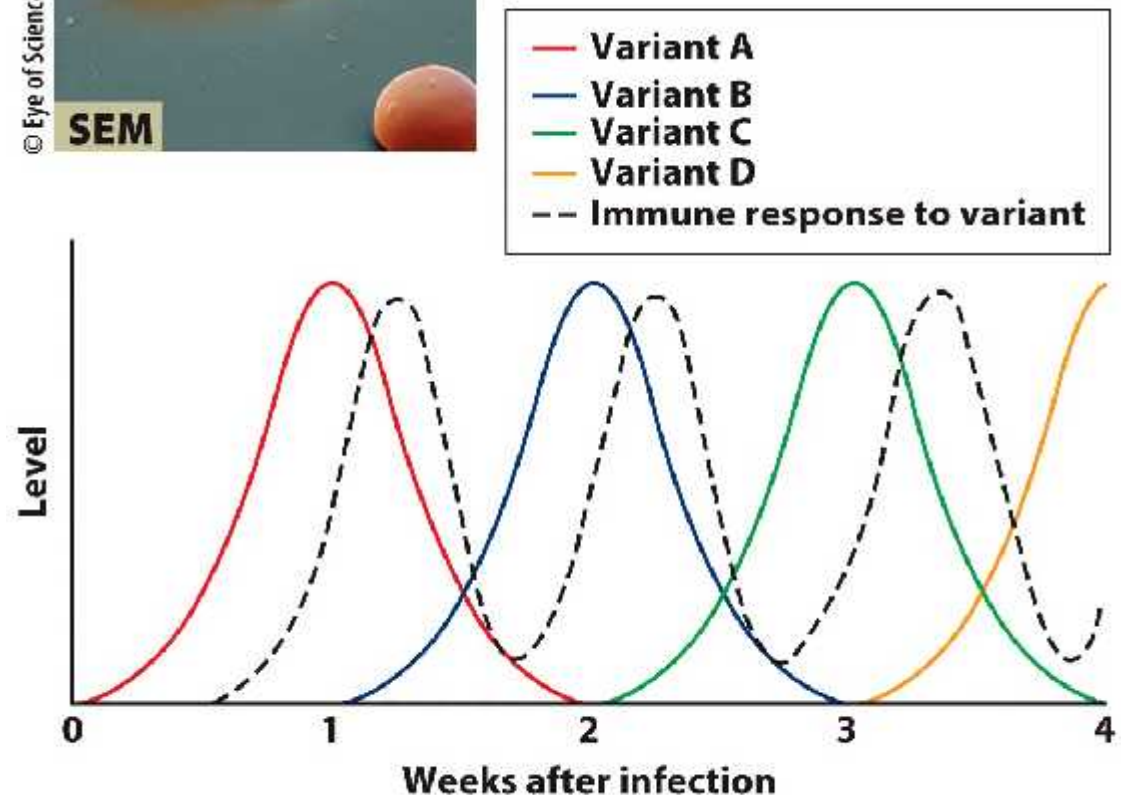 - After attachment and invasion, pathogens must still avoid elimination by host defenses.
  - Some microbes employ antigenic variation, shifting their surface protein structures.
    - *T. brucei*
    - *N. gonorrhoeae*
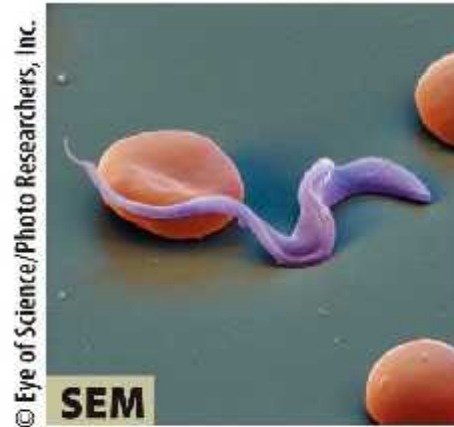
Trypanosoma brucei gambiense
The agent of human sleeping sickness

© Eye of Science/Photo Researchers, Inc.

SEM

— Variant A
— Variant B
— Variant C
— Variant D
- - Immune response to variant

Level

0   1   2   3   4
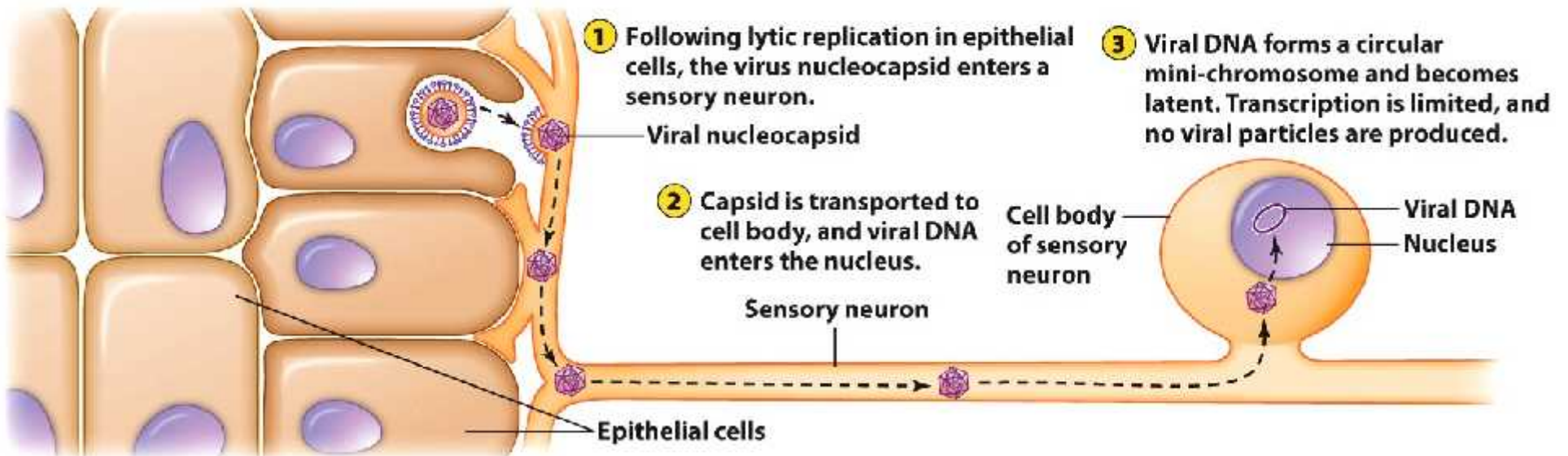
Weeks after infection

- Evading host defenses
  - Antigenic variation isn't the only evasion method.
    - Latency can be used by herpes viruses.
    - Capsules on bacteria make them hard to phagocytose.
    - Some microbes can even replicate inside of phagocytes!

TABLE 18.4 Selected strategies for evading host defenses

Evasion strategy	Example	Function
Antigenic variation	Influenza virus *Trypanosoma brucei*	Avoid antibody-mediated immunity of host
Latency	Herpes simplex virus Epstein Barr virus (EBV)	Avoid recognition by host immune system
Capsule formation	*Streptococcus pneumoniae*	Inhibit phagocytosis
Biofilm formation	*Pseudomonas aeruginosa*	Prevent access to bacterial cells
Replication within macrophages	*Listeria monocytogenes*	Facilitates cell-to-cell spread

- Evading host defenses
  - Latency may be the ultimate evasion method.
    - The virus inserts its genome into host cells.
    - Replication stops.
    - Periodic reactivation may occur.

① Following lytic replication in epithelial cells, the virus nucleocapsid enters a sensory neuron.
— Viral nucleocapsid

② Capsid is transported to cell body, and viral DNA enters the nucleus.

Sensory neuron

③ Viral DNA forms a circular mini-chromosome and becomes latent. Transcription is limited, and no viral particles are produced.

Cell body of sensory neuron

Viral DNA
Nucleus

→ Epithelial cells

Establishment of latency

Virus latency (or viral latency) is the ability of a pathogenic virus to lie dormant (latent) within a cell, denoted as the lysogenic part of the viral life cycle. **Wikipedia**

- Evading host defenses
  - Latency may be the ultimate evasion method.
    - The virus inserts its genome into host cells.
    - Replication stops.
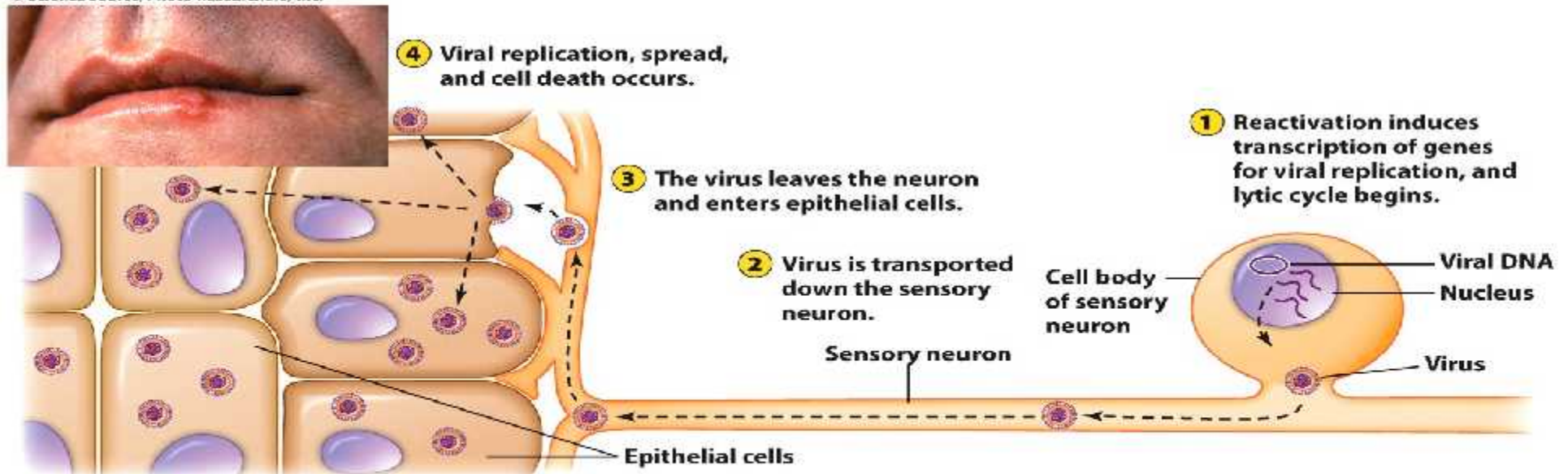   - Periodic reactivation may occur.

④ Viral replication, spread, and cell death occurs.

① Reactivation induces transcription of genes for viral replication, and lytic cycle begins.

③ The virus leaves the neuron and enters epithelial cells.

② Virus is transported down the sensory neuron.

Cell body of sensory neuron

Sensory neuron

Viral DNA

Nucleus

Virus

Epithelial cells

Reactivation and lytic replication

Microbial virulence strategies:

- Evading host defenses
  - Even bacteria exhibit defense mechanisms.
    - Bacteria use restriction endonucleases to digest phage DNA.
    - Phage evasion mechanisms (e.g., RE inhibitors) also exist.

Unmethylated *EcoRI* site of phage DNA gets cut.

EcoRI enzyme

Phage DNA

AATTCTTACGG
GAATGCC

CGGTAG
GCCATCTTAA

Methylated *EcoRI* site of bacterium is protected from digestion.

Bacterial DNA

EcoRI enzyme

CH_3

EcoRI recognition site

CGGTAGAATTCTTACGG
GCCATCTTAAGAATGCC

CH_3
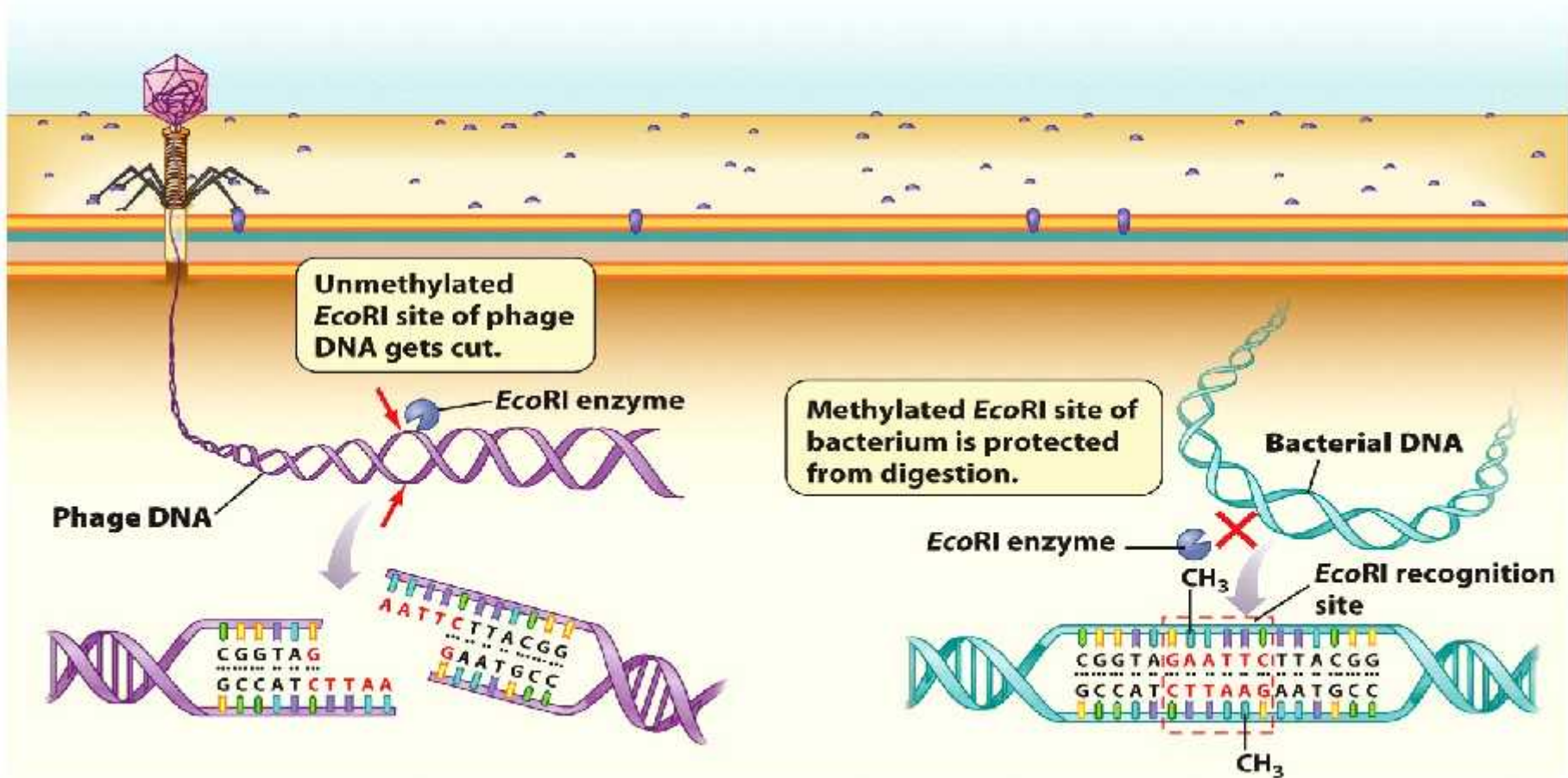
Microbial virulence strategies:

- Pathogenesis
  - Different microbial pathogens cause disease in different ways.
    - Most possess multiple properties that collectively lead to disease induction.
    - Production of toxins is common.
      - **Exotoxins** are proteins produced and secreted that can have negative effects on host cells.
      - **Endotoxins** are a part of the microbial structure itself.
    - Viruses typically don't produce toxins.
      - Instead, their replication induces either cell death or induced cell death (apoptosis) via immune responses to reduce the viral spread.

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The transmission of infectious diseases:

- *How are infectious diseases transmitted?*
- – Routes of transmission
  - – Transmission = Spread of an infectious agent from one host to another
    - May also occur from a pathogen's natural source (reservoir) to a host

The transmission of infectious diseases:

- Routes of transmission
  - Contact
    - Direct = Physical contact between infected/susceptible hosts
    - Indirect = Object carries agent between infected and susceptible individual
      - Object is often a fomite (inanimate object).
  - Fecal-oral
  - Respiratory ("aerosol")
  - Vector-borne
    - Transmitted via another species (e.g., mosquitoes carrying malaria parasites to humans)
  - Sexual transmission

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The transmission of infectious diseases:

- Routes of transmission
  - Horizontal = Transmission of a pathogen between members of a species other than parent to offspring
  - Vertical = Passing of a pathogen from parent to child (often in utero, during birth, or shortly after birth)
    - e.g., HIV
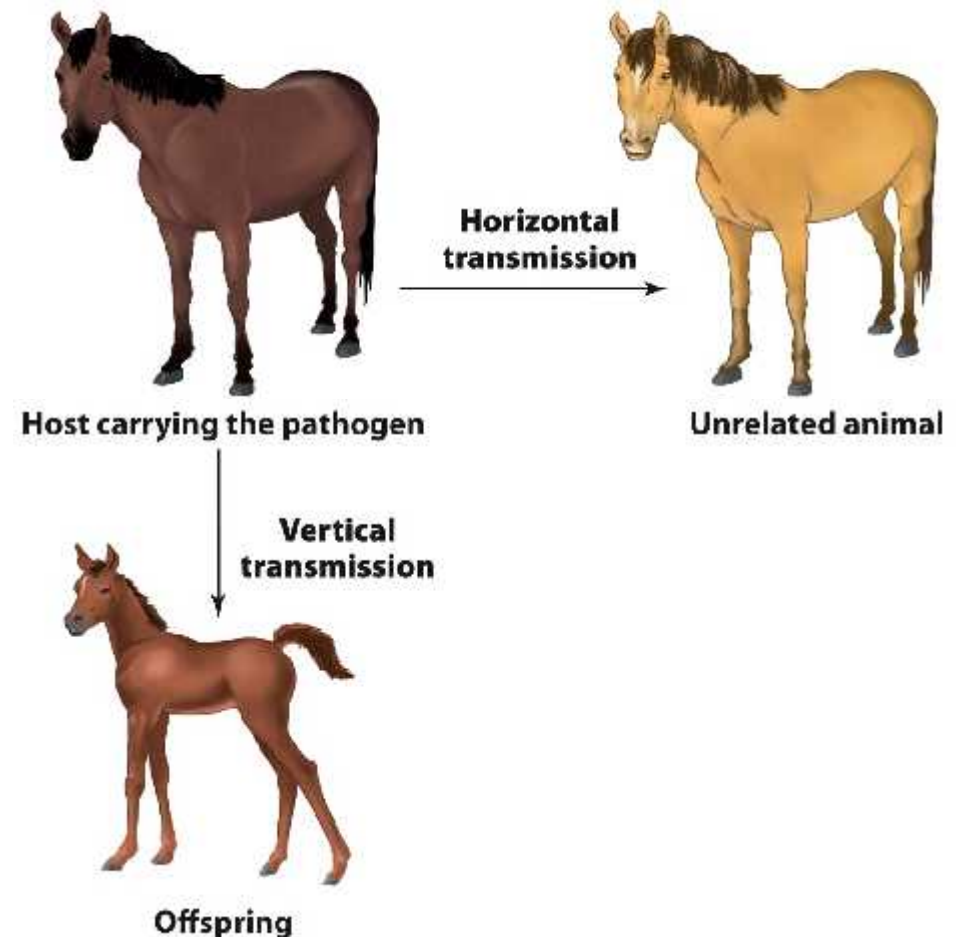
Host carrying the pathogen

Horizontal transmission

Unrelated animal

Vertical transmission

Offspring

The transmission of infectious diseases:

- Routes of transmission
  - Zoonotic transfers = Pathogen moves from its natural (reservoir) host to a human
    - Humans are often "dead-end" hosts, where the pathogen isn't efficiently transferred from person to person.

| TABLE 18.5	Selected zoonotic diseases	
Disease	**Pathogen**	**Animal host(s)**
Rabies	Rabies virus	Many mammals (raccoons, bats, skunks, foxes)
Hantavirus pulmonary syndrome	Hantavirus	Rodents
Ebola hemorrhagic fever	Ebola virus	Unknown (bats?)
Anthrax	*Bacillus anthracis*	Cattle, sheep, goats
Tularemia	*Francisella tularensis*	Rodents, rabbits
Psittacosis	*Chlamydia psittaci*	Many bird species

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The transmission of infectious diseases:
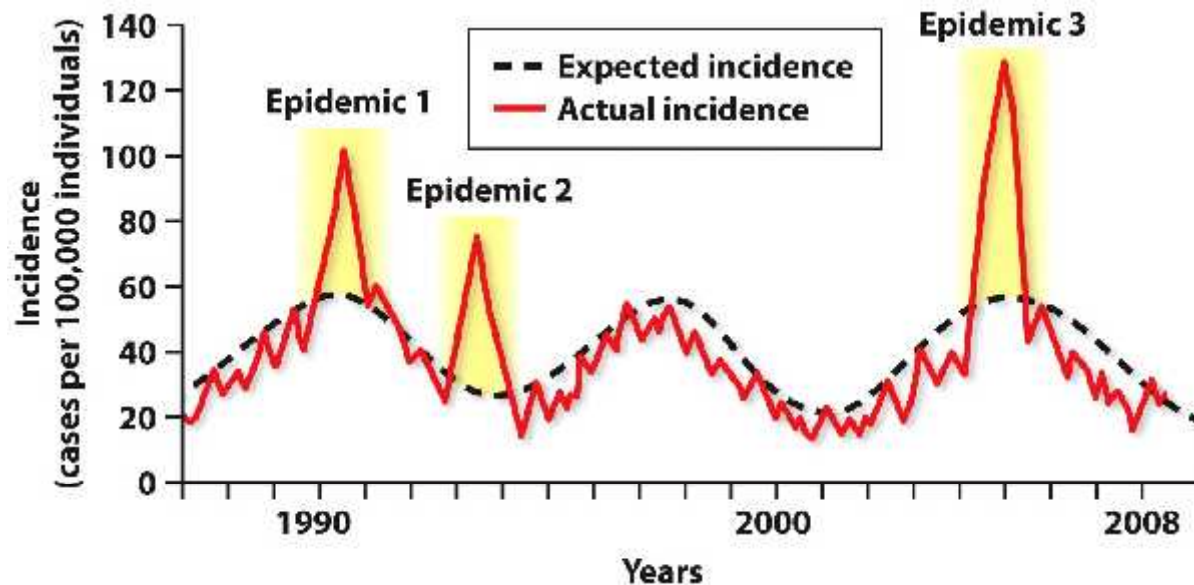
- Patterns of infectious disease
  - Epidemic = Incidence of a disease rises significantly above the normally expected value
  - Outbreak = Unexpected cluster of cases in a short time in a localized population

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- Types of epidemics
  - Common-source epidemics = A single source of infection to which the population is exposed
    - One of the most famous examples was the 1854 cholera outbreak in the **Soho area** of London.
    - John Snow showed the point of contamination was the Broad Street common water pump by tracking cholera cases on a map.

Pumps

Deaths from Cholera

Black: *Microbiology: Principles and Explorations*, copyright 2012, John Wiley & Sons, Inc.
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- Types of epidemics
  - Propagated epidemic = infection passing from one host to another
    - Often results when an infected individual is introduced into a susceptible population
      - Examples include measles, influenza, chickenpox, tuberculosis
    - Typically exhibits a gradual increase in incidence over time
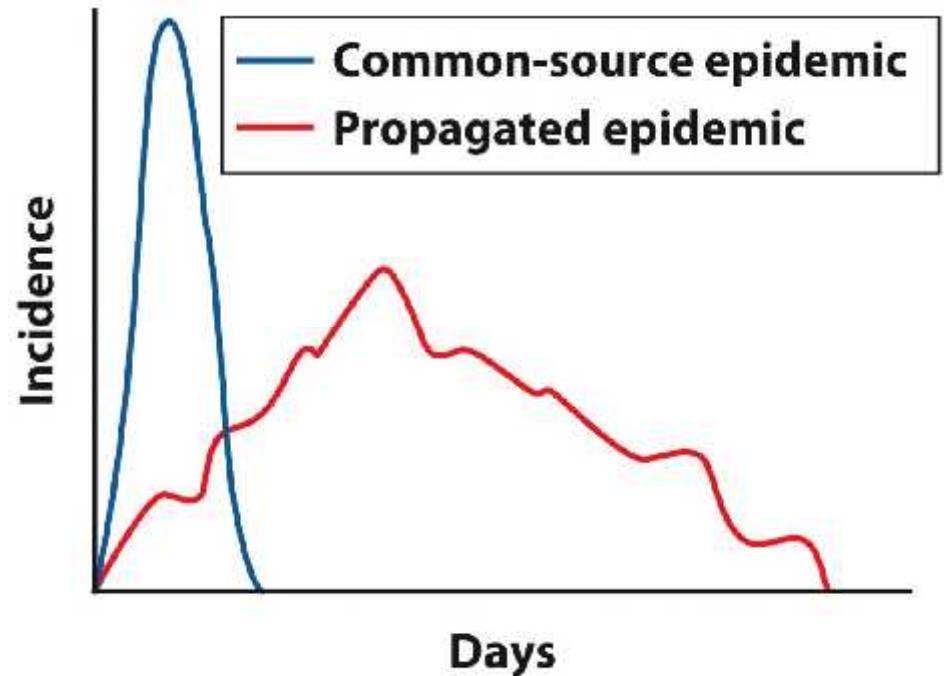

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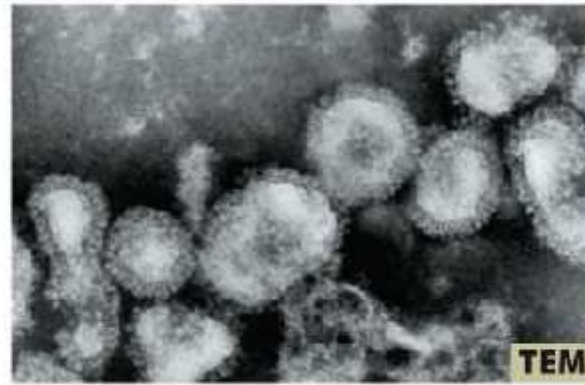# Proving cause and effect in microbial infections:

- How can we determine the cause of an infectious disease?
- Koch's postulates can be used to show a specific microbe causes a specific disease.
  - The cause and effect are proven if
    - The suspected microbe is identified in every person with the disease, but not those without the illness.
    - A pure culture of the suspected microbe is obtained.
    - Experimental inoculation of the suspected microbe into a healthy test host causes the same illness.
    - The suspected microbe is recovered from the experimentally inoculated host organism.

- Koch's postulates in action: Gastric ulcers
  - Ulcers are sores on the lining of the stomach, thought to be caused by excess acid.
  - In the 1980s, researchers isolated a microbe (*Helicobacter pylori*) from ulcerated tissue.
    - By applying classic Koch's postulate rules, this microbe was found to be the causative agent of stomach ulcers.

Esophagus

Ulcers

Stomach

32
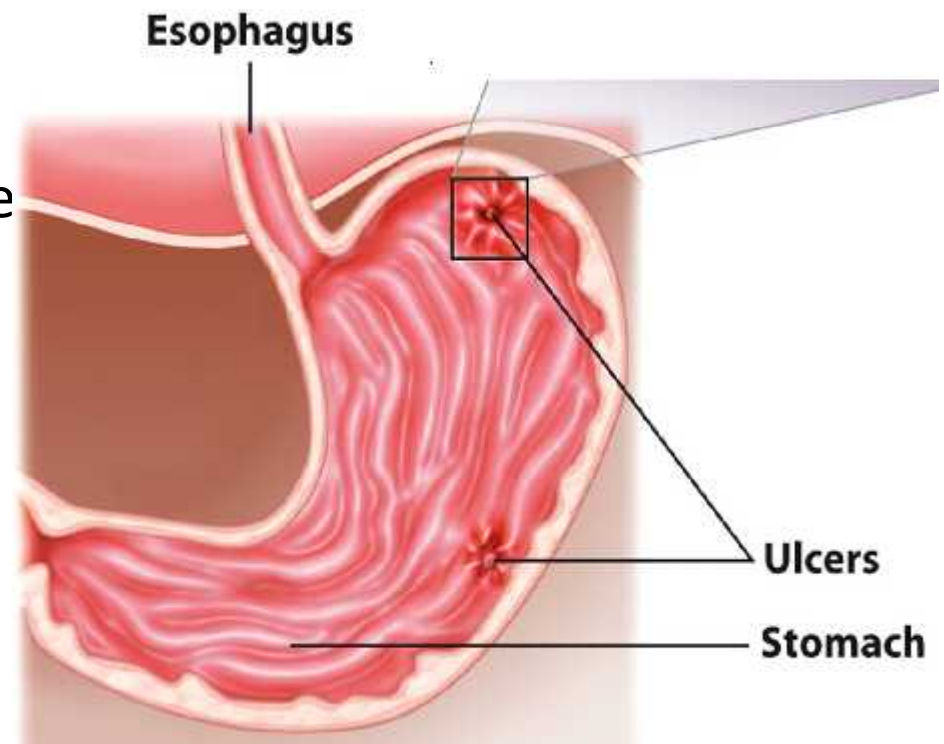
Proving cause and effect in microbial infections:

- Koch's postulates
  - Problem: Not all individuals exhibit the same degree of infection (or may exhibit no infection at all).
    - There is a genetic basis for susceptibility to certain infections.
    - This was proven many years later with inbred mouse strains.

Courtesy Frederick A. Murphy, University of Texas Medical Branch, Galveston

TEM

MHV-A59
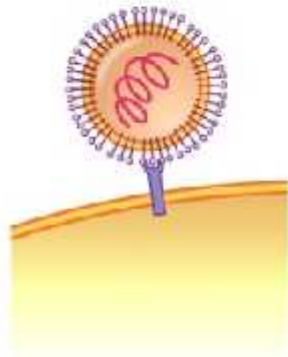
Virus can bind the cell receptor.

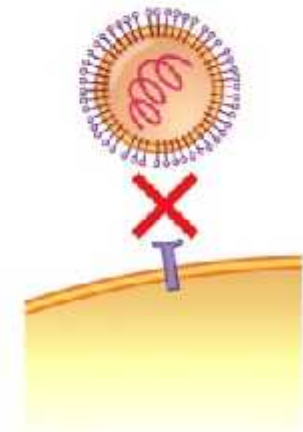Virus cannot bind the variant cell receptor.

Infection and death

No infection

BALB/c mice

SJL/J mice

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Proving cause and effect in microbial infections:

- Molecular Koch's postulates
  - There are several points where the classic postulates may not be ethical or possible to achieve.
  - A more modern take on these "rules" includes adaptations for today's molecular biology tools.
    - The virulence factor should be present in the pathogen.
    - Experimental inactivation of the virulence factor gene should decrease virulence.
    - Reversion of the inactivating change should restore virulence.
    - The virulence factor gene should be expressed during an infection.
    - Immunity to the pathogen must provide protection.

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The evolution of pathogens:

- Microbes becoming more virulent
  - Pathogenic *E. coli*
    - We have *E. coli* in our intestines and it doesn't harm us.
    - Why does some *E. coli* become dangerous?
      - Acquisition of virulence factor genes via horizontal gene transfer
      - *E. coli* O157:H7 produces a toxin derived from *Shigella* species.
      - This toxin allows it to destroy host cells by shutting down protein production, making this strain much more dangerous.

The evolution of pathogens:

- Microbes becoming more virulent
  - Methicillin-resistant *Staphylococcus aureus*
    - Again, *S. aureus* is very common in us and on us.
      - In 1961, reports of MRSA began.
      - Since the 1990s, MRSA infection rates have increased.
    - Selective pressures of antibiotic overuse have led to acquisition of resistance traits against the drugs.
      - A "normal" microbe becomes significantly more dangerous to humans as strains able to resist elimination drugs are selected.

Microbiology for Nursing students

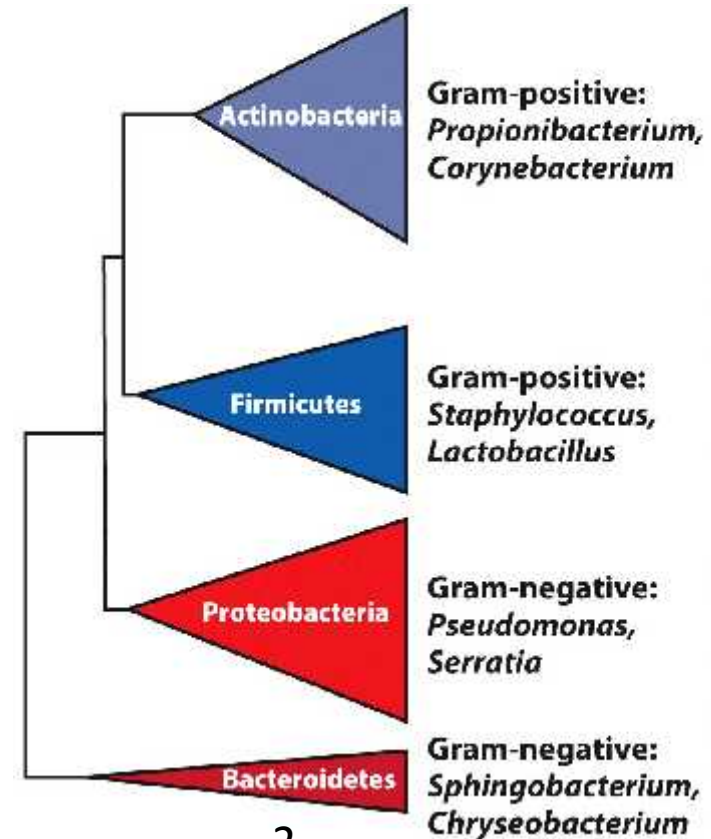## Chapter Five : Bacterial Pathogenesis
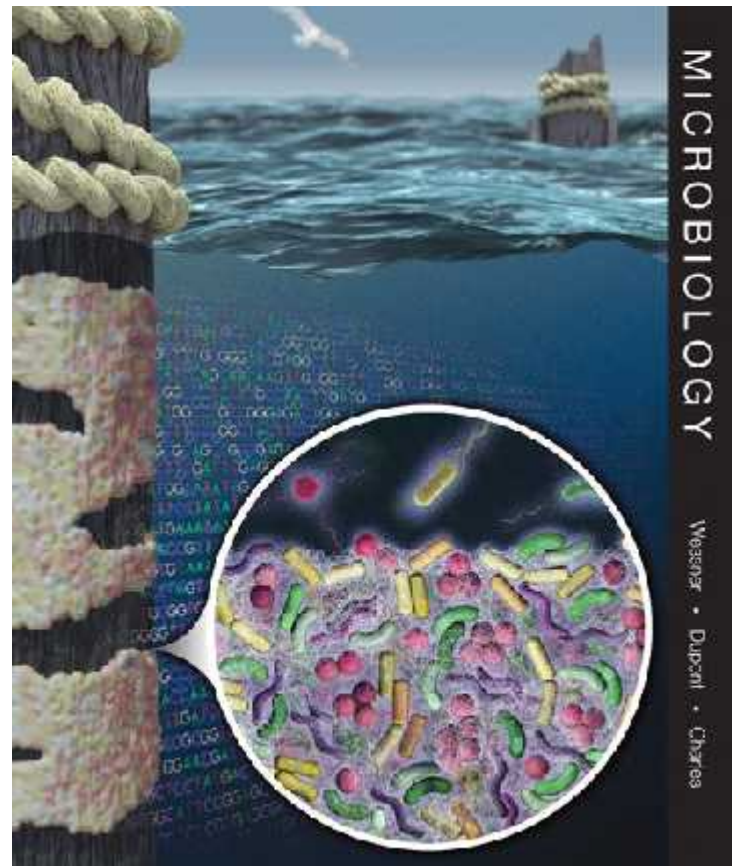
Dr. Sulaiman Alnaimat 2015

- Symbionts of humans: Skin
  - Oil glands and hair follicles form microbe habitat areas.
  - Patterns of bacteria types found on skin are emerging.
  - By crowding out harmful competitors, these microbes may help with disease prevention.

Hair follicle — Epidermis

Sebaceous gland

— Dermis

Sweat gland
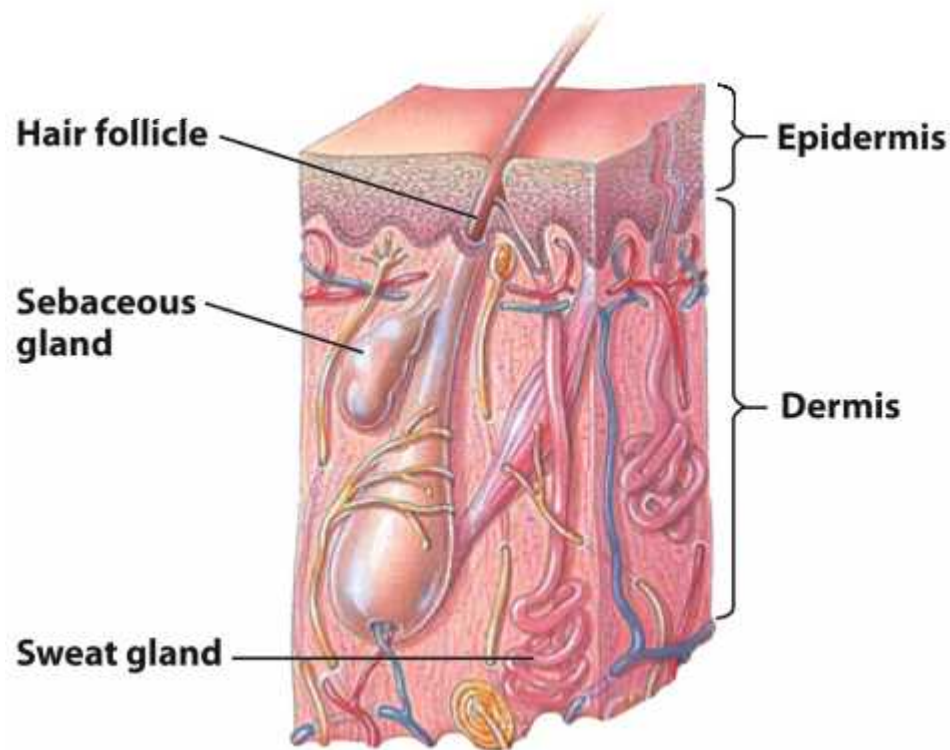
Kuntzman, Tortora: *Anatomy & Physiology for the Manual Therapies*, copyright 2010, John Wiley & Sons, Inc. This material is reproduced with permission of John Wiley & Sons, Inc.

Actinobacteria — Gram-positive: *Propionibacterium, Corynebacterium*

Firmicutes — Gram-positive: *Staphylococcus, Lactobacillus*

Proteobacteria — Gram-negative: *Pseudomonas, Serratia*

Bacteroidetes — Gram-negative: *Sphingobacterium, Chryseobacterium*

Zhan Gao, Chi-hong Tseng, Zhiheng Pei, and Martin J. Blaser, 2007, Proceeding of the National Academy of Sciences 104:2927–2932, National Academy of Sciences, U.S.A.

2

Symbionts of humans
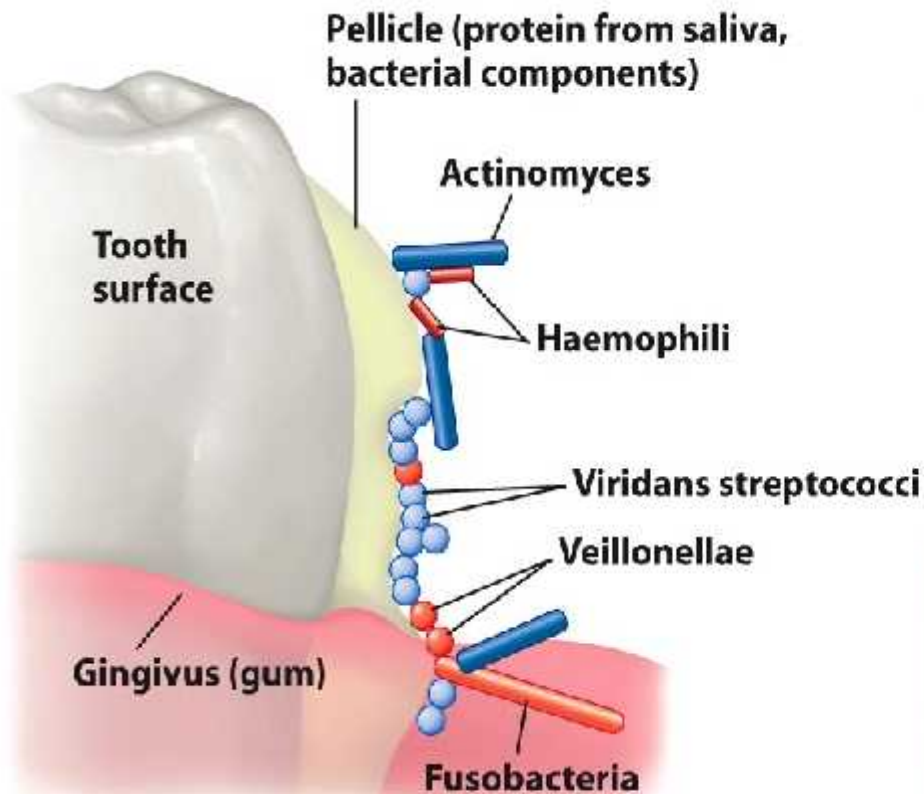
- Symbionts of humans: Vagina
  - Microbial populations here are affected by
    - Proximity to the skin and anus
    - pH of the vaginal tract
    - Moisture levels
    - Age (which often affects hormone levels and pH balance)
  - Common symbionts in this area include
    - *Staphylococcus epidermidis*
    - *E. coli* and *Enterococcus faecalis*
    - *Candida* spp. (a yeast organism)

Symbionts of humans

- Symbionts of humans: Oral Cavity
  - Second in colonization only to the colon
  - Provides warm, moist, nutrient-rich area for microbes
  - Over 700 bacterial species detected in the mouth
    - Mainly by 16S rRNA gene analysis
    - Over half of the 700 cannot be cultured.
  - Common members include
    - Gram-positive streptococci (e.g., *Streptococcus mutans*)
    - Anaerobic *Fusobacterium* spp. below the gumline
    - Anaerobic *Porphyromonas gingivalis* deep in plaque deposits

- Symbionts of humans: Oral Cavity
  - Plaque biofilm buildup is a major cause of cavities (dental caries) and periodontal disease.

A. Early colonization
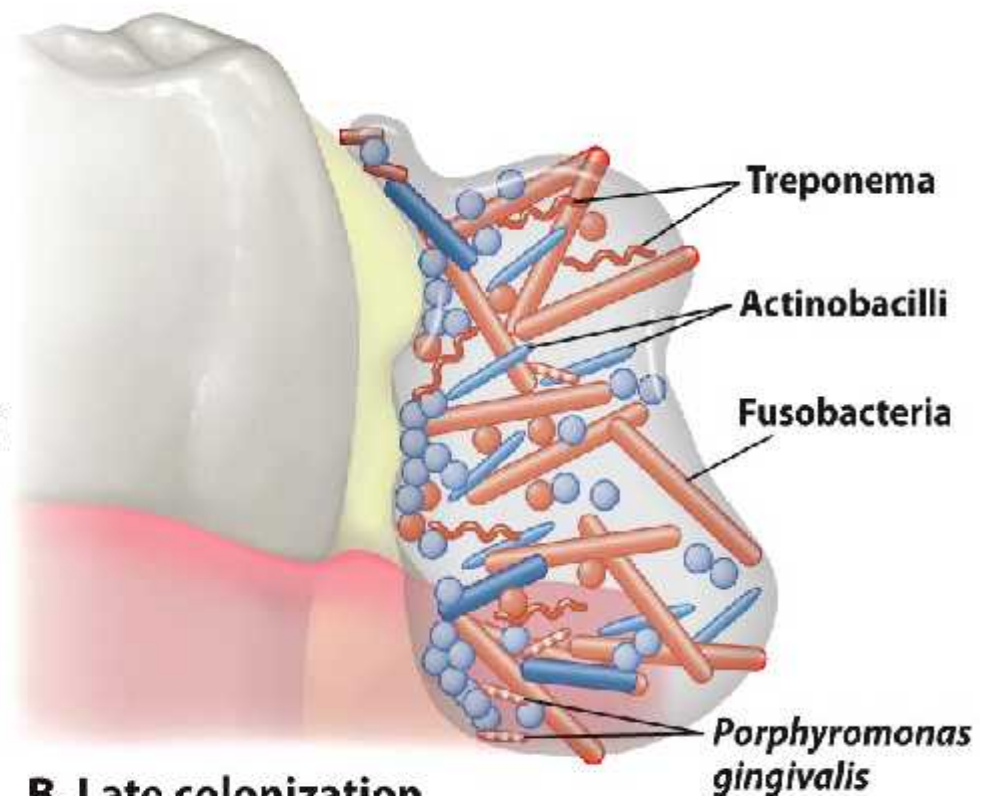
B. Late colonization

5

- Symbionts of humans: Oral Cavity
  - Plaque biofilm buildup is a major cause of cavities (dental caries) and periodontal disease.

Healthy gums
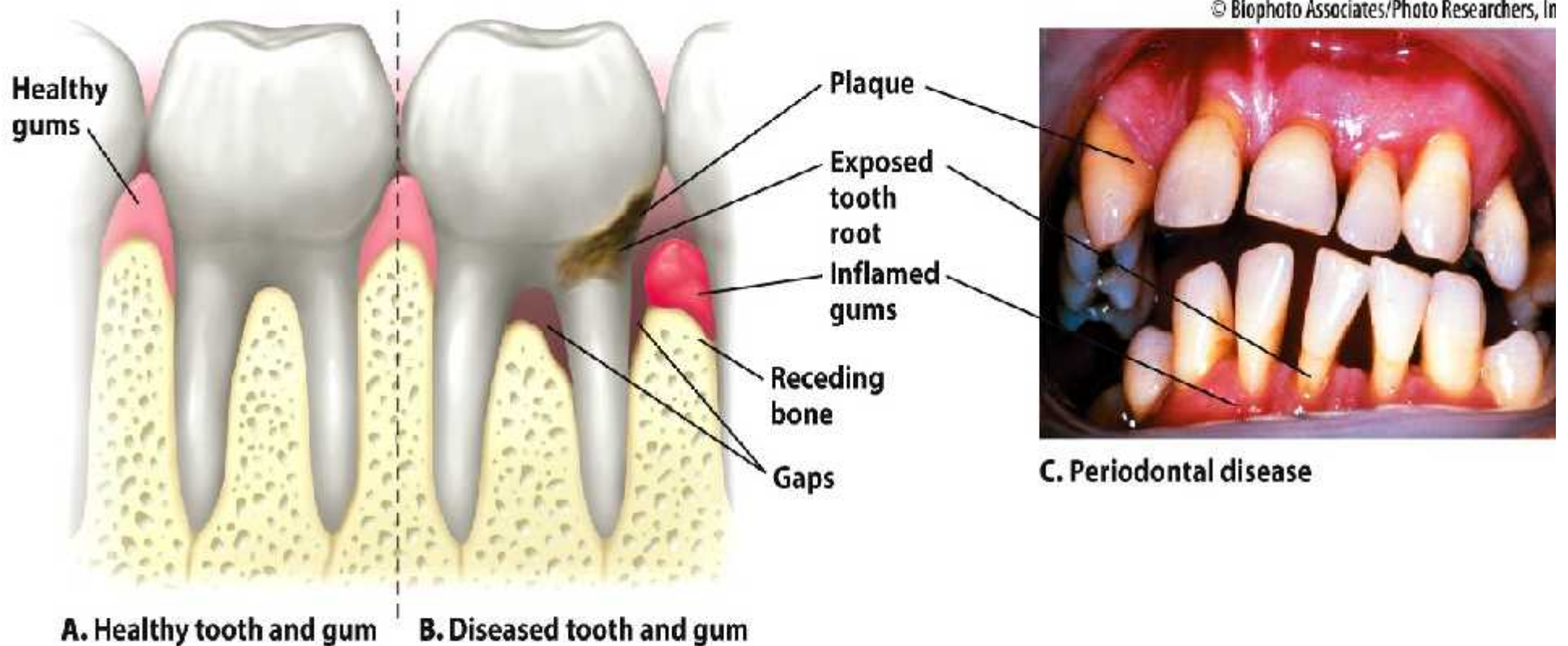
Plaque

Exposed tooth root

Inflamed gums

Receding bone

Gaps

A. Healthy tooth and gum   B. Diseased tooth and gum

C. Periodontal disease

© Biophoto Associates/Photo Researchers, In

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- Symbionts of Humans: Digestive Tract
  - Could include between 800 and 2,500 (or more) bacteria
  - Most likely also includes a variety of bacteriophages
  - Anatomy/diet (and other factors) lead to dynamic bacterial population changes moving along the length of the tract

Major families (ileum)

Lactobacillaceae
Pasteurellaceae
Enterobacteriaceae

Major phyla (colon)

Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria

Major families (colon)

Clostridiaceae
Bacteroidaceae
Prevotellaceae
Enterobacteriaceae
Lactobacillaceae
Streptococcaceae
Bifidobacteriaceae
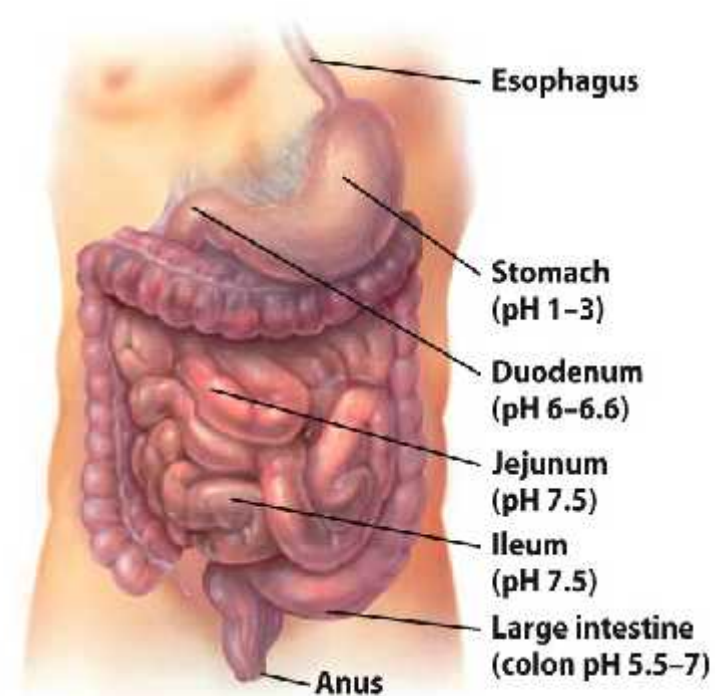
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Cecum
Appendix

Esophagus
Stomach (pH 1–3)
Duodenum (pH 6–6.6)
Jejunum (pH 7.5)
Ileum (pH 7.5)
Large intestine (colon pH 5.5–7)
Anus

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- # Symbionts of Humans: Digestive Tract

TABLE 17.2 Major groups and metabolic activities of intestinal microorganisms of humans

Major group	Characteristics	Metabolic activities
Phylum Firmicutes 　Class Clostridia	Gram-positive anaerobic spore-forming bacilli	Fermentation of starch, glucose to butyrate and acetate
Phylum Bacteroidetes 　Family Bacteroidaceae, Family Prevotellaceae	Gram-positive non-spore-forming rods or cocci	Fermentation of plant-derived carbohydrates
Phylum Proteobacteria 　Family Enterobacteriaceae	Gram-negative non-spore-forming bacilli	Fermentation of various sugars
Phylum Actinobacteria	Gram-positive non-spore-forming rods or cocci	Fermentation of starch to lactate
Domain Archaea 　*Methanobrevibacter smithii*	Only archaeon consistently present in the gut	Methane production from CO_2 and H_2

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- Symbionts of Humans: Digestive Tract
  - Do these microbes attach to the human tissues?
    - It doesn't appear so (except for pathogenic microbes).
    - Instead, they seem to form a biofilm along the mucus

Mucus layer

Lumen

Intestinal epithelium

Colonizing symbiotic microorganisms (biofilm)
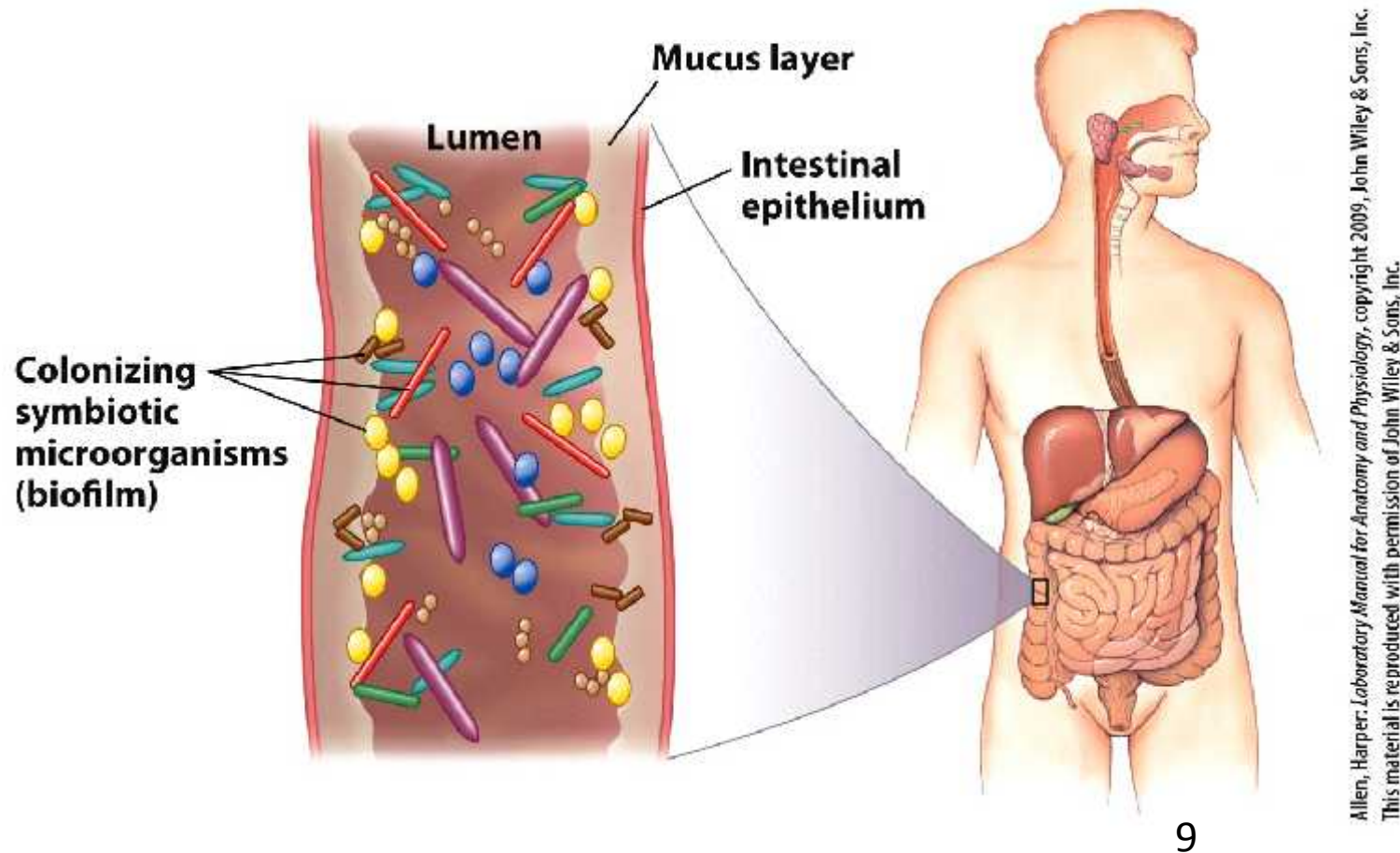
- **Symbionts of Humans: Digestive Tract**
  - What are the benefits of this relationship to humans?
    - Some microbes produce vitamins that we absorb (Vitamin K).
    - Some microbes assist in digestion of materials in food prior to absorption.
    - Proper immune function is also a benefit of their presence.
      - Intestinal microbes can "crowd out" pathogenic microbes.
      - More benign members of the intestinal flora can also "prime" the immune system, keeping it ready in case we ingest a more dangerous microbe related to our normal intestinal flora.
      - Also, routine exposure to these microbes may keep our immune system in balance, preventing autoimmune diseases and allergies (the "hygiene hypothesis").

Probiotics – Do They Work?

– Probiotics are live microbes that, when ingested, may provide a beneficial effect to the human body.

– Results of studies on their effectiveness have varied widely.

- They may be beneficial in lactose intolerance, antibiotic-induced diarrhea and in childhood illness-related diarrhea.

- BUT can a small addition to a VERY LARGE number already in the intestines actually have an effect?

- Also, can they survive in the stomach to even reach the intestines?

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- Pathogenesis = Processes used by pathogens to produce disease
- Bacterial pathogens have evolved mechanisms to
  - **Attach** to our surfaces and tissues
  - **Damage** tissues to obtain nutrients and replicate
  - **Avoid** host immune responses
- This chapter will focus on the first two aspects of pathogenesis, and to a lesser extent on the third aspect.

Bacterial virulence factors:

- *What are common features of bacterial pathogens?*
  - Virulence factors = Pathogen products that enhance their ability to cause disease

- **Attachment factors**
  - Some of the first virulence factors a host will encounter are those the pathogen uses to attach.
    - Fibronectin-binding proteins
    - Fimbriae
    - Various membrane-associated molecules (capsules)
    - Specialized proteins for attachment

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TABLE 21.1 **Some major virulence factors of selected pathogens**

Organism	Disease	Virulence factor	Action
Bordetella pertussis	Whooping cough	Fimbriae	Attachment
		Pertussis toxin	Disrupts cell ion balance
		Invasive adenylate cyclase	Disrupts cell ion balance
Escherichia coli O157:H7	Hemorrhagic colitis and kidney failure	Intimin	Attachment
		Tir	Receptor for attachment
		Type III secretion system	Injects Tir for attachment
		Shiga toxins	Stops translation in host cells
Helicobacter pylori	Gastritis, ulcers	Urease	Neutralizes gastric acid
		Vacuolating cytotoxin A (VacA)	Host cell death, inflammation
		Flagella	Transport through mucus
		Cytotoxin-associated antigen (CagA)	Disrupts host cell cytoskeleton
		CagA type IV secretion system	Injects CagA
Neisseria gonorrhoeae	Gonorrhea	Fimbriae	Attachment and immune evasion
		IgA protease	Destruction of IgA antibody
		LOS (a form of endotoxin)	Evokes inflammatory damage
Streptococcus pneumoniae	Pneumonia, meningitis	Capsule	Anti-phagocytic
		Pneumolysin	Forms pores in host cells
		Autolysin	Lysis of bacterial cell to release peptidoglycan (produces inflammation)
Streptococcus pyogenes	Various skin, throat, and systemic infections	Capsule	Anti-phagocytic
		M protein	Prevents binding by antibody
		Hyaluronidase	Degrades connective tissue
		Streptokinase	Degrades fibrin clots

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Bacterial virulence factors:

- Attachment factors

  1. **Fibronectin-binding proteins**
     - Fibronectin = Large plasma glycoprotein in plasma and extracellular matrix
     - Since it's everywhere in the body, it's a prime target for pathogen binding.

  2. **Fimbriae = Specialized pili with an adhesive tip**
     - The tip binds to a specific receptor on a host cell.
     - Acts as a "probe" to get beyond the repulsing negative charge on the host cell
     - Can be altered by some microbes to evade immunity

  3. **Special adherence proteins Tir and Intimin**

Bacterial virulence factors:
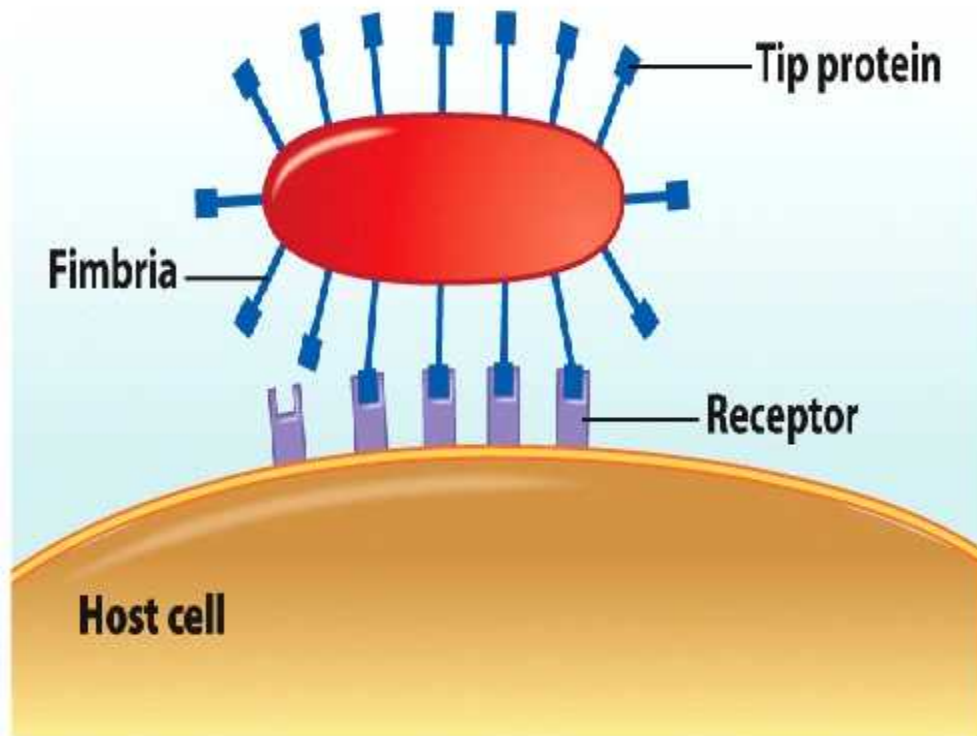
A. Attachment by fimbriae
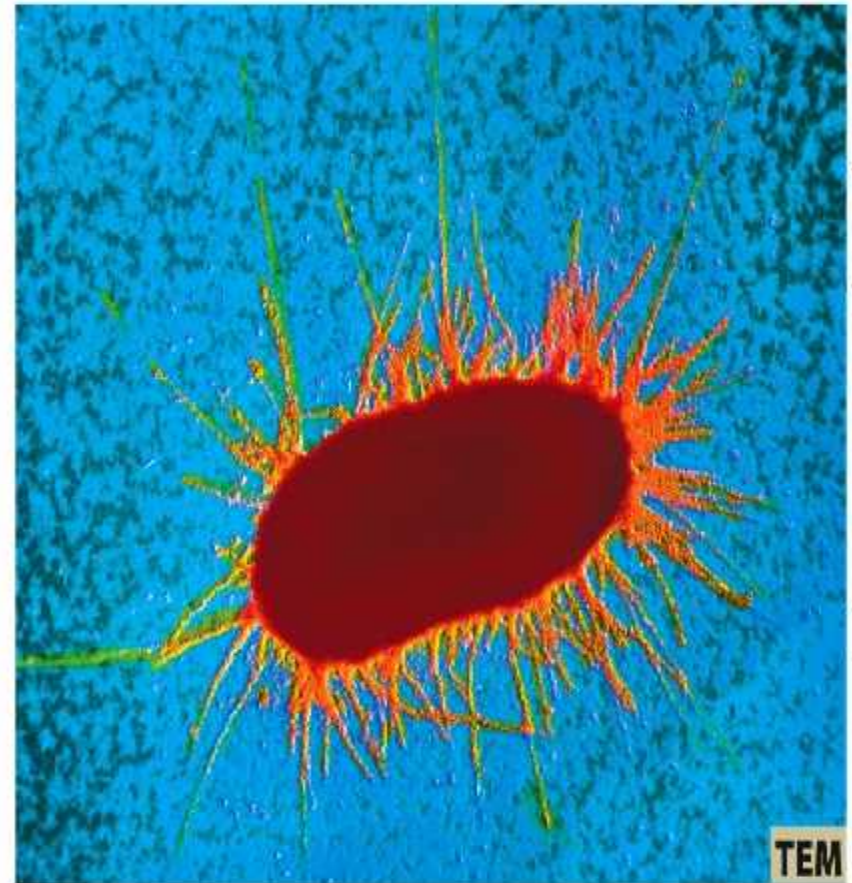
B. Fimbriae of E. coli

16
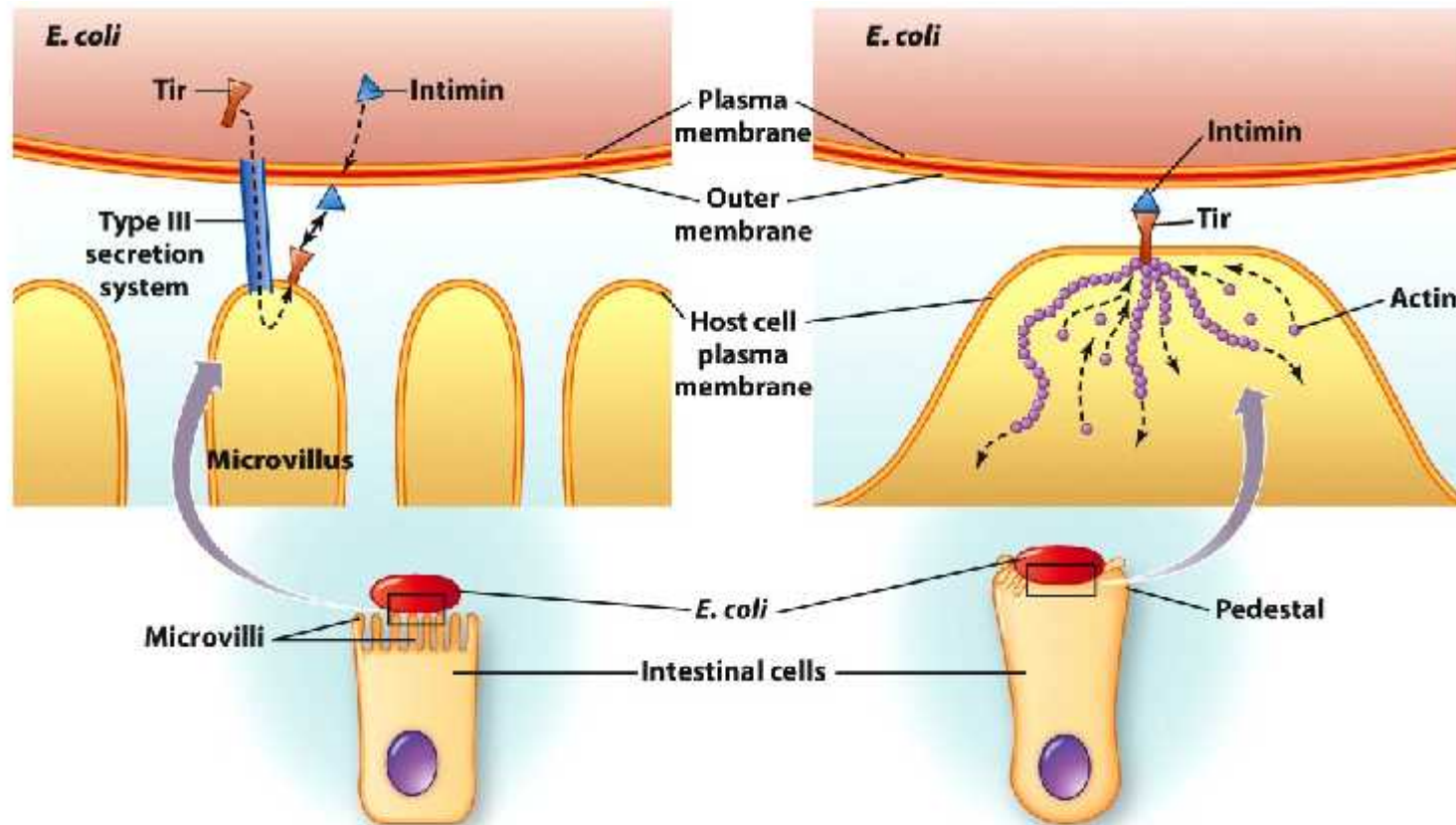
A. Injection of Tir into host cell

B. Intimate attachment and pedestal formation
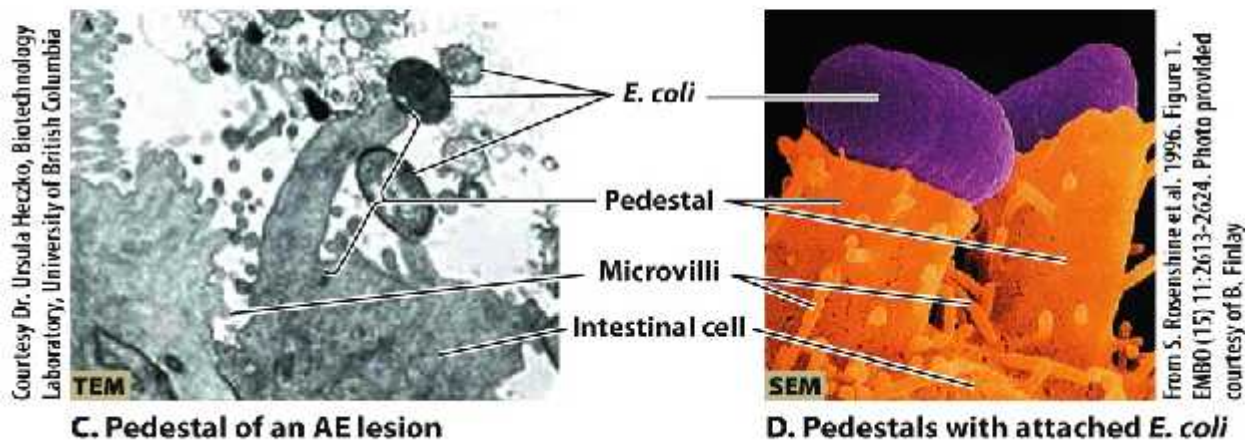
C. Pedestal of an AE lesion

D. Pedestals with attached *E. coli*

17

Bacterial virulence factors:

- Bacterial toxins
  - Endotoxins are a part of the cell wall structure and induce inflammatory responses.
    - Lipopolysaccharide (LPS) on Gram-negative cells
    - Lipoteichoic acid (LTA) on Gram-positive cells
  - Exotoxins are released outside the producing cell.
    - A-B toxins: B subunit binds to host cell receptor. A subunit has a negative action inside the cell.
    - Cytolysins: Act on cell membranes
    - Superantigens: Nonspecifically stimulate T cells to secrete large amounts of cytokines

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Bacterial virulence factors:

- Bacterial toxins
  - Endotoxin and lipoteichoic acid
    - Lipopolysaccharide (LPS) is the most common endotoxin.
    - It has three parts:
      - O-antigen (often strain-specific, can be used for serotyping)
      - Core polysaccharide
      - Lipid A (the inflammatory inducing portion)

Plasma membrane   Outer membrane

Cytoplasm

Periplasm

Lipopolysaccharide (LPS)

Lipooligosaccharide (LOS)

Lipid A	Core polysaccharide	O-antigen
• Unusual fatty acids • Toxic activity • Links LPS to outer membrane	• Various sugars with side chains • Genus or species-specific	• Many repeating units of polysaccharide • Strain-specific • Target of immune response • Used for serotyping

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Bacterial virulence factors:

- Bacterial toxins
  - Endotoxin and lipoteichoic acid
    - Similar in nature to LPS but found on Gram-positive cells
    - Recognized by TLRs
    - Capable of inducing inflammation

Biological effects of endotoxin and lipoteichoic acid

TABLE 21.4

Effect	Protective activity
Fever	Inhibition of pathogen replication Increase in immune cell activities
Complement activation	Lysis by MAC formation Induction of inflammation
Inflammation	Transport of immune cells and molecules to site of infection
B cell proliferation	Antibody production
IFN-γ expression from T cells	Activation of macrophages and NK cells
Stimulation of clotting cascade	Prevention of pathogen spread

Bacterial virulence factors:

- Bacterial toxins: A-B toxins
  - B portion binds to a receptor on a host cell.
  - A portion has enzymatic activity inside host cells.
  - Many pathogens secrete A-B toxins.

A subunit has toxic enzymatic activity.

B subunit binds cell receptor.

Diphtheria toxin
Tetanus toxin
Botulinum toxin

Cholera toxin
Shiga toxins

Pertussis toxin

Corynebacterium diphtheriae

A subunit

B subunit

Diphtheria toxin receptor

Host cell

Endosome

① Released diphtheria toxin binds to cellular receptors by B subunit

② Endocytosis

③ Acidification allows dissociation of B subunit from receptor and channel formation

H^+

Channel

④ A subunit enters cytoplasm through channel

Enzymatic A subunit

⑤ A subunit inactivates translation factor EF2, abolishing translation

Elongation factor 2 (EF2)   Modified EF2

Bacterial virulence factors:

- Bacterial toxins:
A-B toxins

Shiga toxin

rRNA

A

Protein synthesis

Ribosomal RNA cleavage

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Botulinum toxin

A

SNARE proteins

Acetylcholine secreted → Muscle contraction.

Acetylcholine not secreted → Muscle contraction. → Flaccid paralysis

Tetanus toxin

A

Synapto-brevin

Glycine or GABA secreted → Acetylcholine not secreted → Muscle contraction halted.

Glycine or GABA not secreted → Acetylcholine secreted → Muscle contraction halted. → Spastic paralysis

SNARE protein cleavage

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TABLE 21.5 A-B toxins, source, activity, target cells, and disease

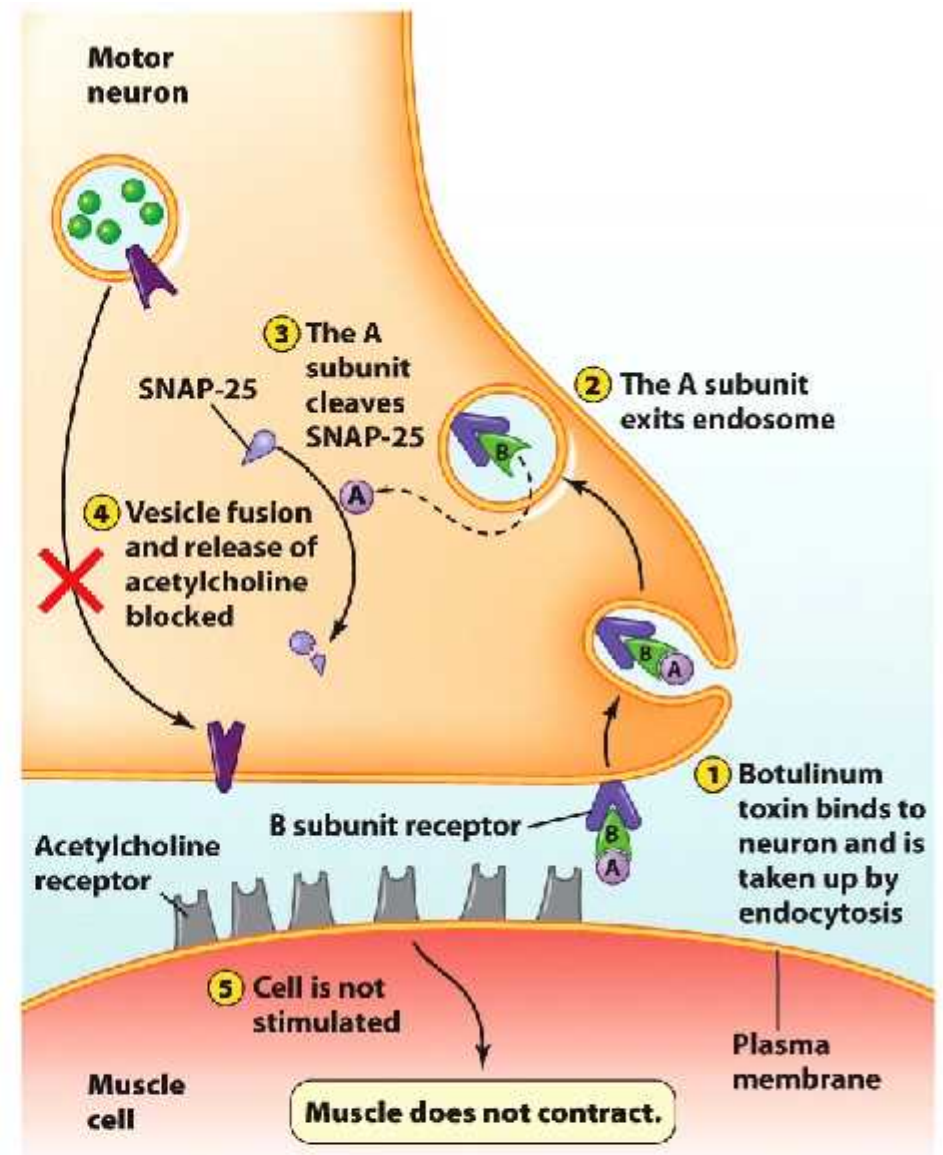| Toxin	Bacterium	Activity	Target cells (receptor-restricted)	Disease
Diphtheria toxin	*Corynebacterium diphtheriae*	ADP-ribosylation of EF2; inhibition of protein synthesis	Upper respiratory tract, heart, nerves, kidney	Diphtheria
Botulinum toxin	*Clostridium botulinum*	Cleaves SNARE proteins needed for release of the neurotransmitter acetylcholine	Motor neurons	Botulism
Cholera toxin	*Vibrio cholerae*	ADP-ribosylation of G_s protein; increases cAMP levels; cellular ion disruption	Cells of the intestinal tract where the bacterium colonizes	Cholera
Invasive adenylate cyclase	*Bordetella pertussis*	Increases cAMP levels; cellular ion disruption	Ciliated cells of the respiratory tract where the bacterium colonizes	Whooping cough (pertussis)
LT (heat labile)	Enterotoxigenic *E. coli* strains	ADP-ribosylation of G_s protein; increases cAMP levels; cellular ion disruption	Cells of the intestinal tract where the bacterium colonizes	Diarrhea
Pertussis toxin	*Bordetella pertussis*	ADP-ribosylation of G_i protein; increases cAMP levels; cellular ion disruption	Ciliated cells of the respiratory tract where the bacterium colonizes	Whooping cough (pertussis)
Shiga toxins	*Shigella dysenteriae* and enterohemorrhagic *E. coli*	Cleaves host ribosomal RNA; inhibition of cell protein synthesis	Kidney, liver, neurons	Hemolytic uremic syndrome (HUS)
Tetanus toxin	*Clostridium tetani*	Cleaves SNARE protein synaptobrevin needed for release of neuroinhibitors	Inhibitory neurons	Tetanus (lockjaw)

23

Vesicle containing acetylcholine — Motor neuron — **Synaptobrevin**

① Synaptobrevin binds to SNAP-25

② SNAP-25 complex binds to syntaxin — SNAP-25

③ The vesicle fuses with the plasma membrane

Syntaxin

Released acetylcholine

④ Acetylcholine binds to receptors on muscle cell

Acetylcholine receptor

⑤ Cell is stimulated

Plasma membrane — Muscle cell

Muscle contracts.

A. Normal function of motor neuron

Motor neuron

③ The A subunit cleaves SNAP-25 — SNAP-25

② The A subunit exits endosome

④ Vesicle fusion and release of acetylcholine blocked

B subunit receptor

Acetylcholine receptor

① Botulinum toxin binds to neuron and is taken up by endocytosis

⑤ Cell is not stimulated

Muscle cell — **Plasma membrane**
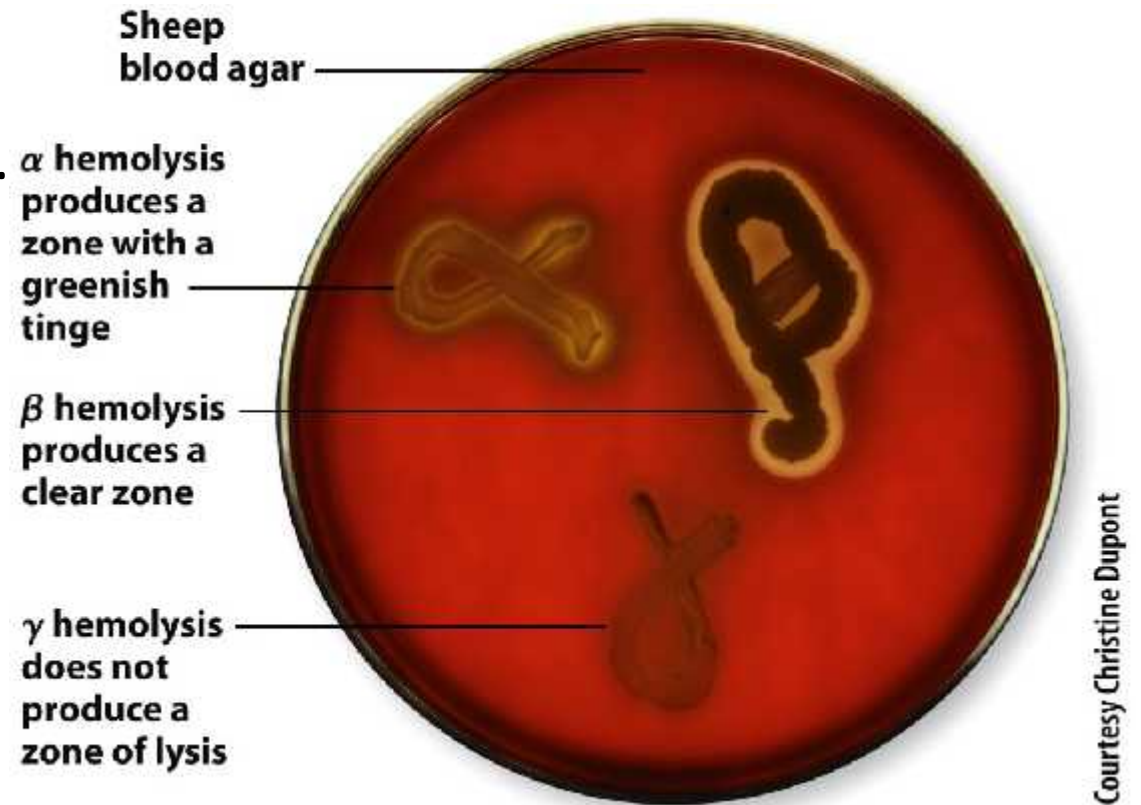
Muscle does not contract.

B. Action of botulinum toxin type A on motor neuron
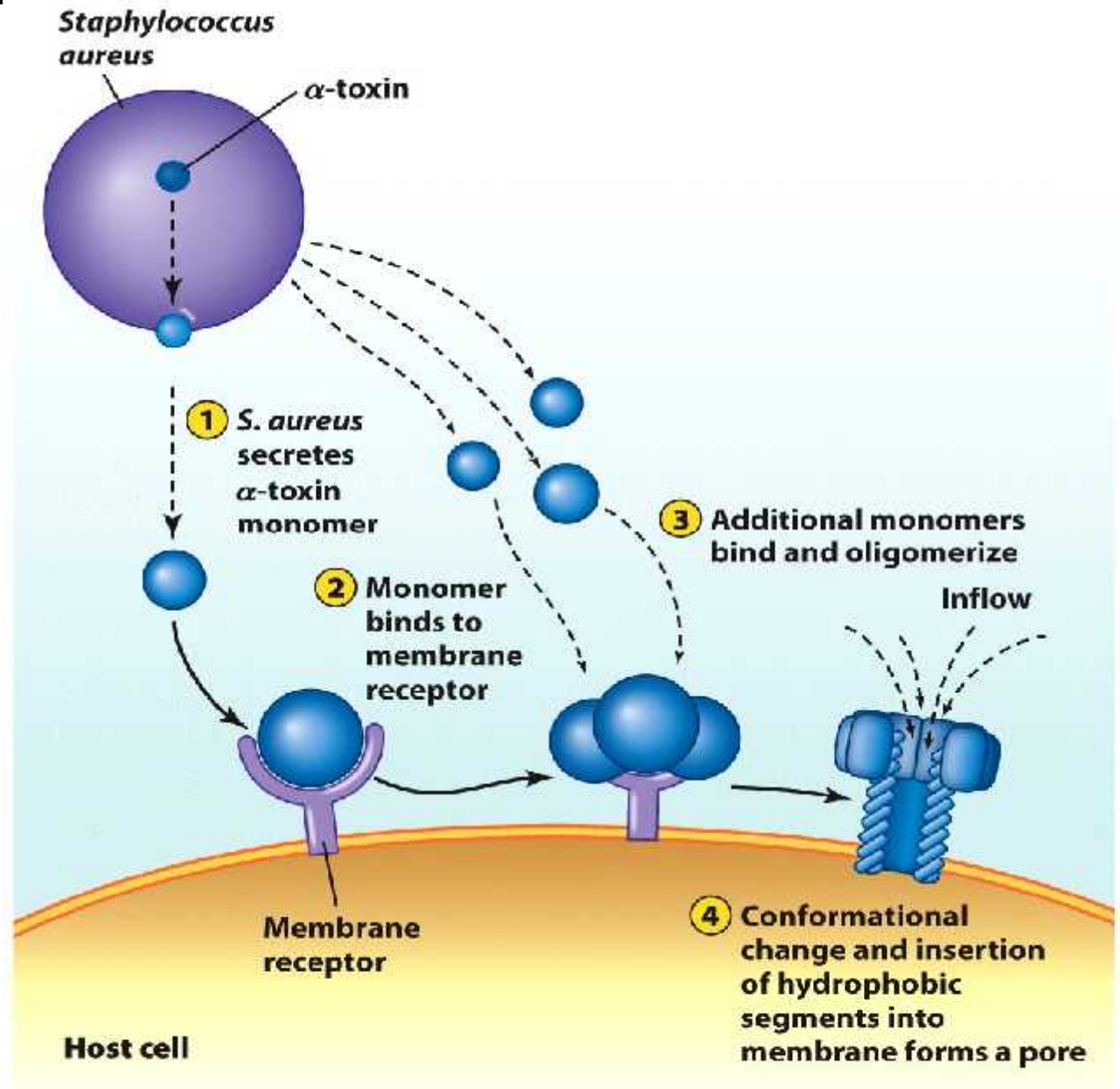
24

- Bacterial toxins: Cytolysins
  - Work on plasma membranes of cells, often forming pores or degrading phospholipids
  - Hemolysins are a classic example, lysing red blood cells.
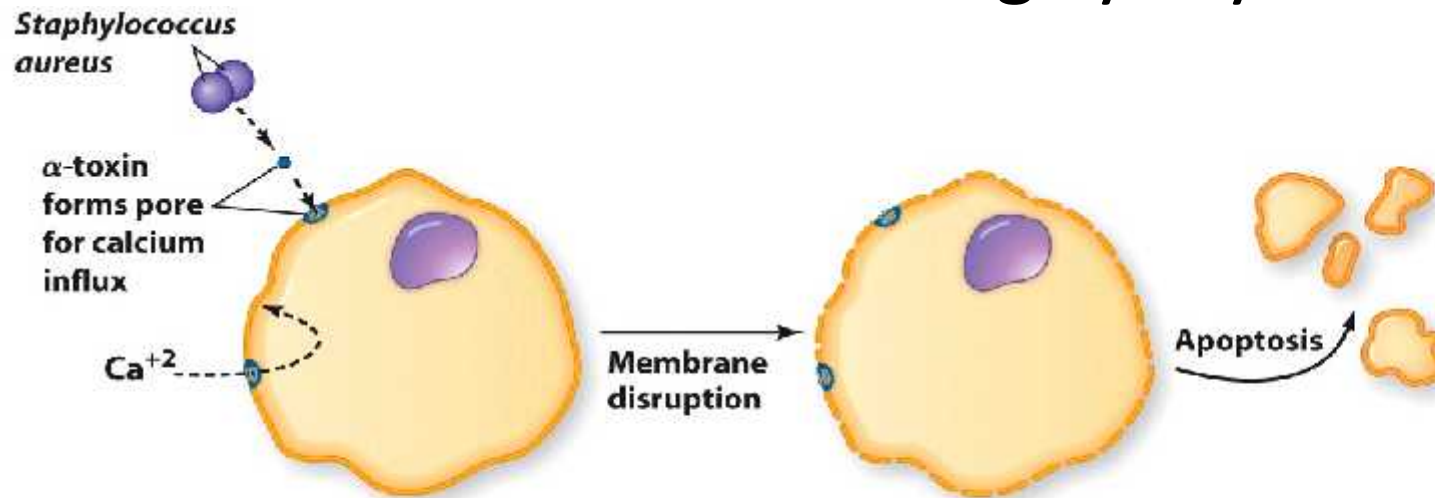   - Lysis pattern can be used as an identifier.

Sheep blood agar

α hemolysis produces a zone with a greenish tinge

β hemolysis produces a clear zone

γ hemolysis does not produce a zone of lysis

Courtesy Christine Dupont

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Bacterial virulence factors:

- Bacterial toxins: Pore-forming cytolysins

Staphylococcus aureus

α-toxin

1. S. aureus secretes α-toxin monomer

2. Monomer binds to membrane receptor

3. Additional monomers bind and oligomerize

Inflow

4. Conformational change and insertion of hydrophobic segments into membrane forms a pore

Membrane receptor

Host cell

- Bacterial toxins: Pore-forming cytolysins

Staphylococcus aureus

α-toxin forms pore for calcium influx

Ca^{+2}

Membrane disruption

Apoptosis

A. Action of *Staphylococcus aureus* α-toxin

Listeria monocytogenes

Listeriolysin

H$^+$

Endosome acidification activates toxin to form pores

Bacterial replication

Endosome membrane disruption

B. Action of *Listeria monocytogenes* listeriolysin
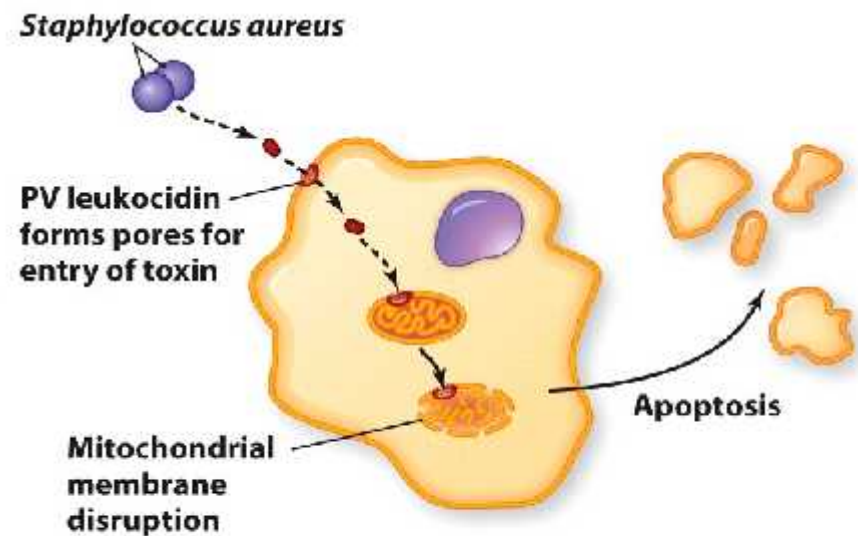
Staphylococcus aureus

PV leukocidin forms pores for entry of toxin

Mitochondrial membrane disruption

Apoptosis

C. Action of *Staphylococcus aureus* PV leukocidin

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Bacterial virulence factors:
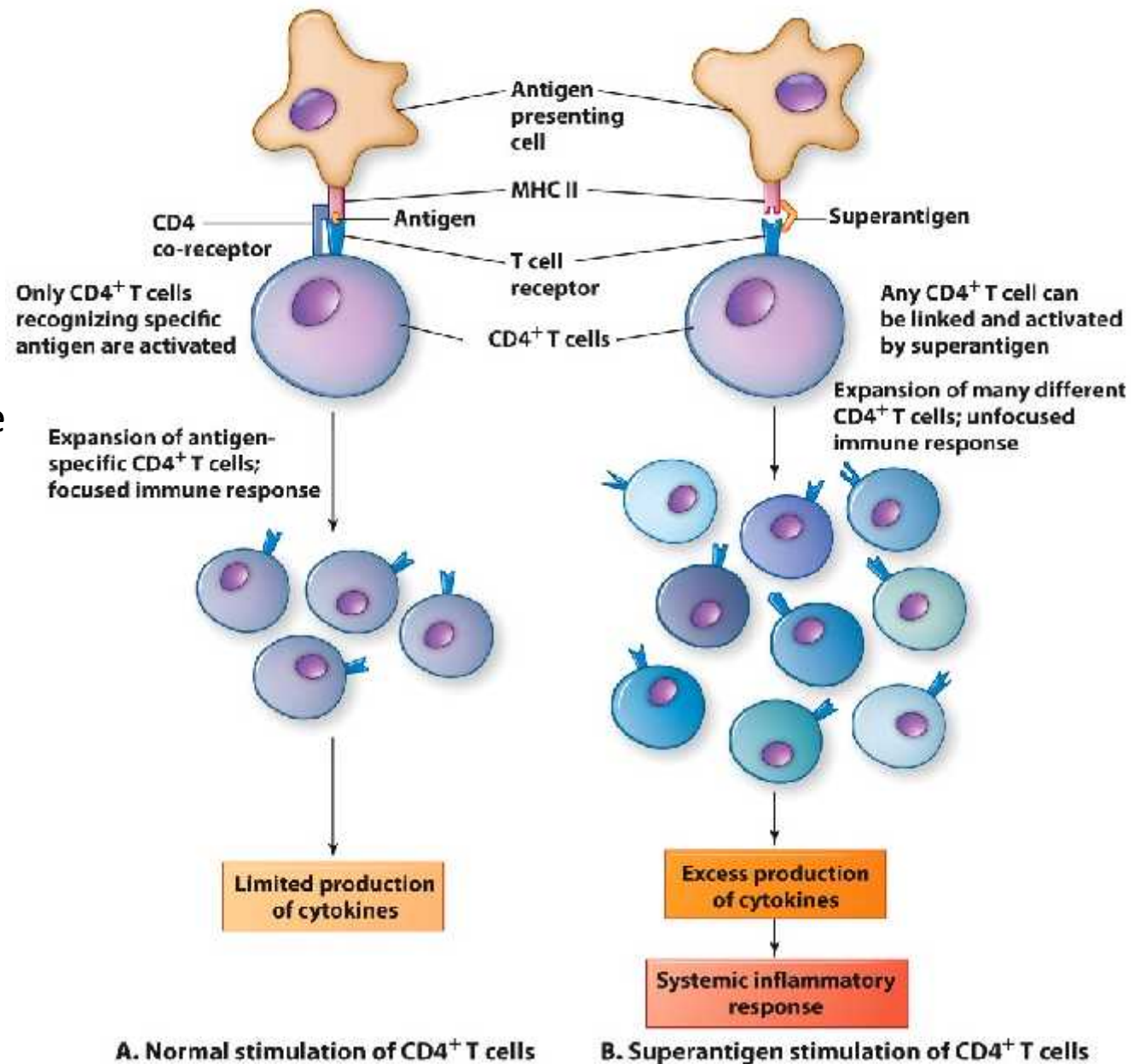

Scott Camazine/Photo Researchers, Inc.

- Bacterial toxins: Phospholipase cytolysins
  - Lecithinases degrade phospholipids.
    - *Clostridium perfringens* α-toxin is a lecithinase associated with gas gangrene.
    - Perfringolysin (also produced in gas gangrene) is a pore-forming cytolysin, however.

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- Bacterial toxins: Superantigens
  - Exotoxins that act on helper T cells
    - Cause a massive release of nonspecific cytokines
    - Induce a systemic inflammatory response

Antigen presenting cell

MHC II

CD4 co-receptor

CD4 — Antigen

Superantigen

T cell receptor

Only CD4⁺ T cells recognizing specific antigen are activated

CD4⁺ T cells

Any CD4⁺ T cell can be linked and activated by superantigen

Expansion of antigen-specific CD4⁺ T cells; focused immune response

Expansion of many different CD4⁺ T cells; unfocused immune response

Limited production of cytokines

Excess production of cytokines

Systemic inflammatory response

A. Normal stimulation of CD4⁺ T cells
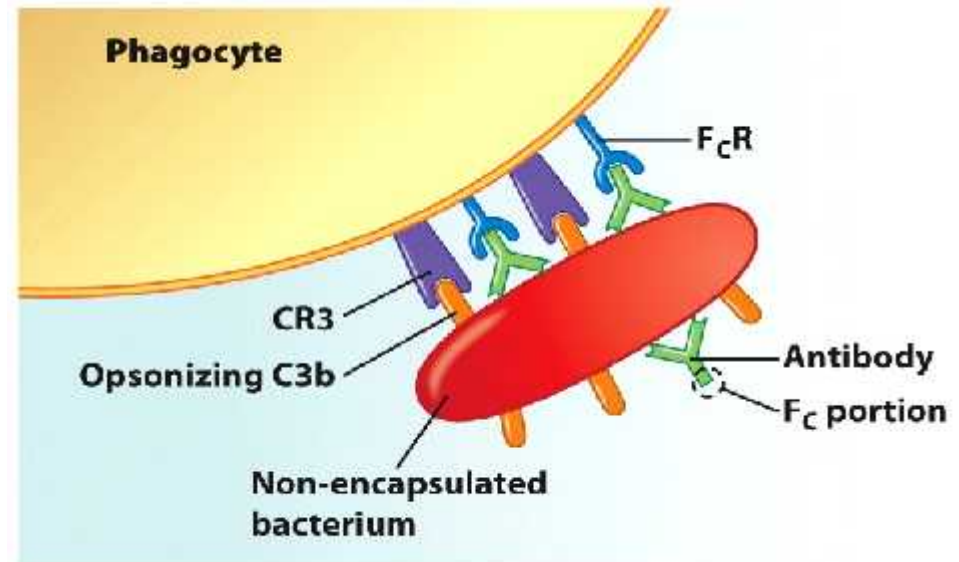
B. Superantigen stimulation of CD4⁺ T cells

Bacterial virulence factors:

- Bacterial toxins: Superantigens AND endotoxins!
  - Each is capable of inducing inflammation individually.
    - Inducing random inflammation confuses coordination of immune responses that would work to eliminate pathogens.
  - Together, they can act synergistically and induce shock and death.

Degree of inflammation
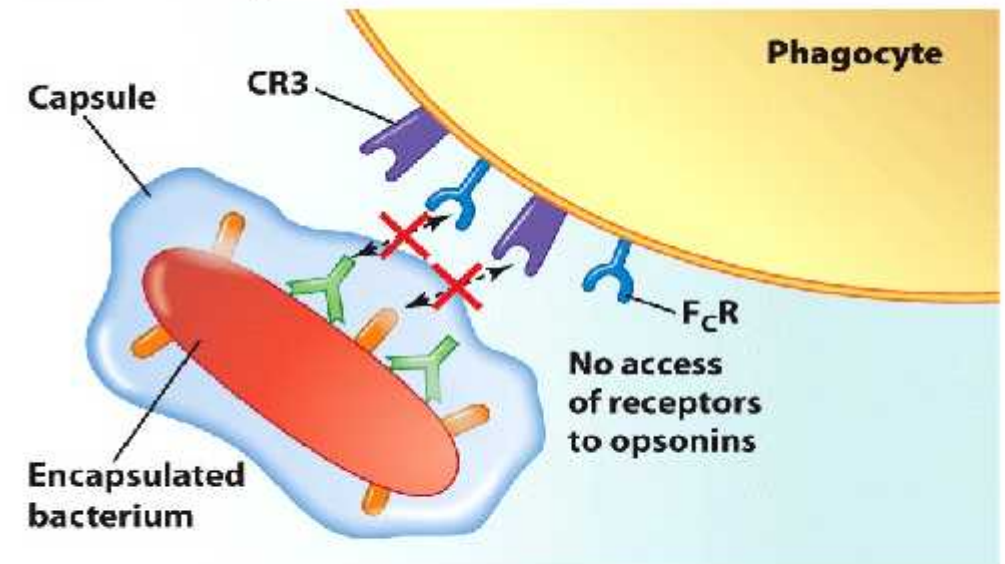
Superantigen

Endotoxin

30
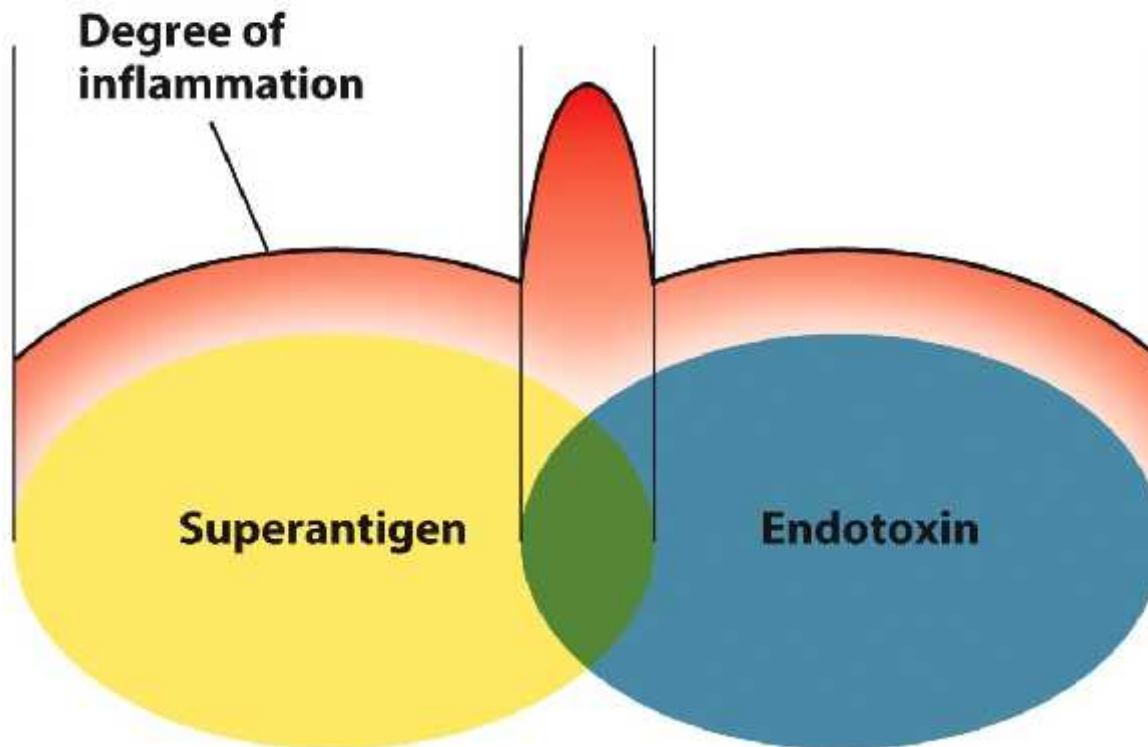
Bacterial virulence factors:

- Capsules
  - Extracellular loose matrix of polysaccharides
  - Provide attachment and immune evasion mechanisms for pathogens
    - Blocks opsonization, interfering with phagocytosis
    - Blocks antibody/complement
    - Reduced entry into endocytic pathway lessens antigen presentation.
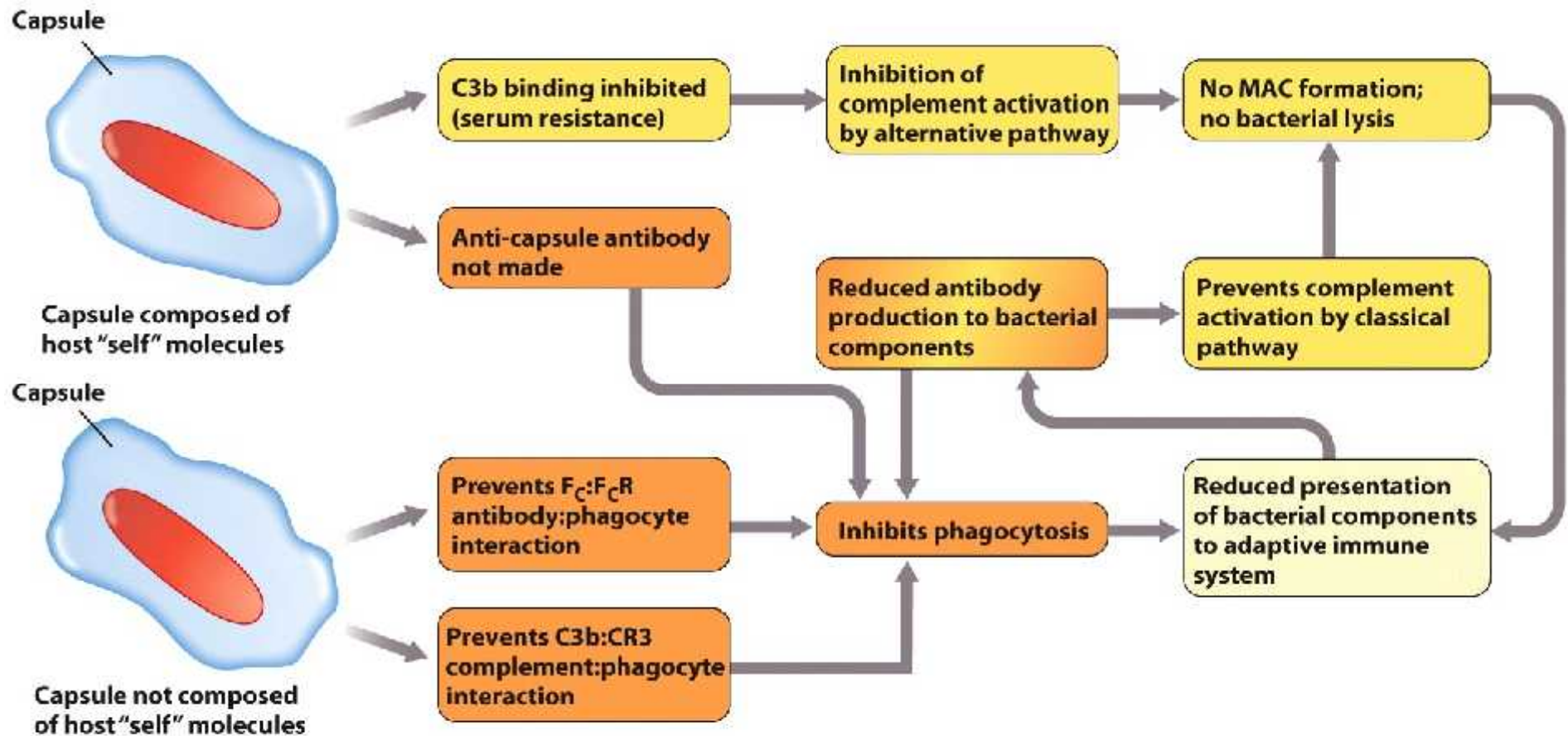   - Mimics "self" molecules, preventing stimulation of antibody/complement responses

A. Non-encapsulated bacterium

Phagocyte — FcR, CR3, Opsonizing C3b, Non-encapsulated bacterium, Antibody, Fc portion

B. Encapsulated bacterium

Capsule, CR3, Phagocyte, FcR, No access of receptors to opsonins, Encapsulated bacterium

Bacterial virulence factors:

Capsule

Capsule composed of host "self" molecules

Capsule

Capsule not composed of host "self" molecules

C3b binding inhibited (serum resistance)

Inhibition of complement activation by alternative pathway

No MAC formation; no bacterial lysis

Anti-capsule antibody not made

Reduced antibody production to bacterial components

Prevents complement activation by classical pathway

Prevents F_C:$F_C R$ antibody:phagocyte interaction

Inhibits phagocytosis

Reduced presentation of bacterial components to adaptive immune system

Prevents C3b:CR3 complement:phagocyte interaction

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Bacterial virulence factors:

- Iron-binding proteins
  - Iron is a limiting nutrient for pathogens.
  - They require mechanisms to get and keep it.
    - Siderophores = Iron-binding molecules that compete with host iron-binding proteins
    - Proteins that can bind host iron-binding proteins, stealing the iron for the bacteria
    - Lowering pH at the site of infection, impairing host iron-binding protein activities
    - Production of cytolysins that lyse host cells to get their iron stores

Survival in the host: Strategies and consequences

- How do bacterial pathogens cause disease?
  - Remember, to cause disease, three processes must be carried out by a pathogen:
    1. Gain access to host tissues
    2. Overcome host defenses
    3. Get nutrients

  - To examine this further, two pathogens will be explored in detail:
    - *Streptococcus pyogenes*
    - *Mycobacterium tuberculosis*

Survival in the host: Strategies and consequences

- Pathogen study 1: *Streptococcus pyogenes*
  - Opportunistic pathogen that produces a variety of diseases
  - It possesses numerous virulence factors.
    - Removing one factor doesn't prevent pathogenicity.
  - Some strains can induce post-streptococcal sequelae.
    - Glomerulonephritis
    - Rheumatic heart disease

Diseases produced by *Streptococcus pyogenes*

Disease	Description
Rheumatic heart disease and acute rheumatic fever © Mark Nielsen	Inflammatory sequelae following pharyngeal infection with SPE-producing strains. Ongoing fever, pain in joints, and fatigue. Can affect skin, joints, central nervous system, and heart. Pathogenesis is thought to be due to autoimmune processes brought on by streptococcal antigens, such as M5 (see Figure 21.25). Permanent damage to heart valves is known as rheumatic heart disease.
Glomerulonephritis 	Inflammatory sequela following *S. pyogenes* infection. Pathogenesis is thought to involve autoimmune processes that damage glomeruli of kidneys. The condition is not limited to *S. pyogenes* infection.

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- Pathogen study 1: *Streptococcus pyogenes*
  - Access to host tissues
    - Primary residence is the pharynx.
    - Attachment factors include fimbriae and other surface components attaching to fibronectin and collagen.
    - Normally kept in check by normal microbiota, but if things change, it may expand its population.
    - Physical damage to skin or mucosa may allow direct access to deeper tissues.

- **Pathogen study 1: *Streptococcus pyogenes***
  - Overcoming host defenses
    - Capsules prevent phagocytosis.
    - Capsules inhibit antibody/complement binding.
    - M proteins can bind antibodies by the F_C region.
    - Several methods for preventing complement activation are present.
  - Collectively, these deter immune responses long enough for the pathogen to expand its population
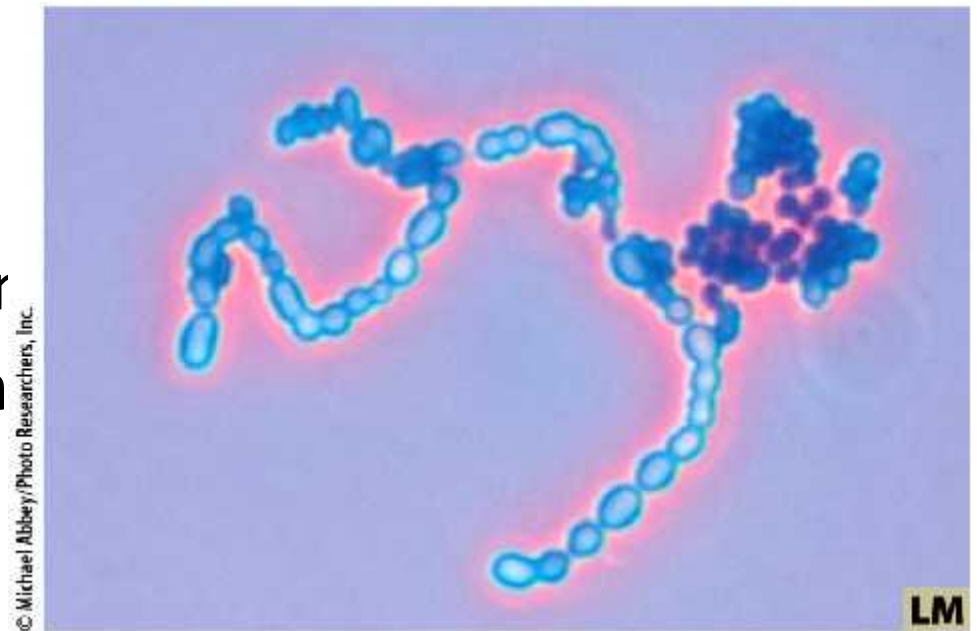
Survival in the host: Strategies and consequences

- **Pathogen study 2: *Mycobacterium tuberculosis***
  - Causes tuberculosis, characterized by destruction of lung tissue
  - Can occur in other areas of the body
  - All members of the genus have an unusual cell wall containing mycolic acids
    - Helps them resist common staining methods
    - All members use similar tactics to induce disease.

- **Pathogen study 2: *Mycobacterium tuberculosis***
  - Access to host tissues
    - Spread by inhalation of aerosols from infected individuals
    - Bacterium replicates inside phagosomes of lung macrophages.
      - This protects the cells from complement-mediated lysis and antibodies.
      - The bacteria actually encourage phagocytosis via opsonization with complement proteins C3b and mannose-binding lectin.
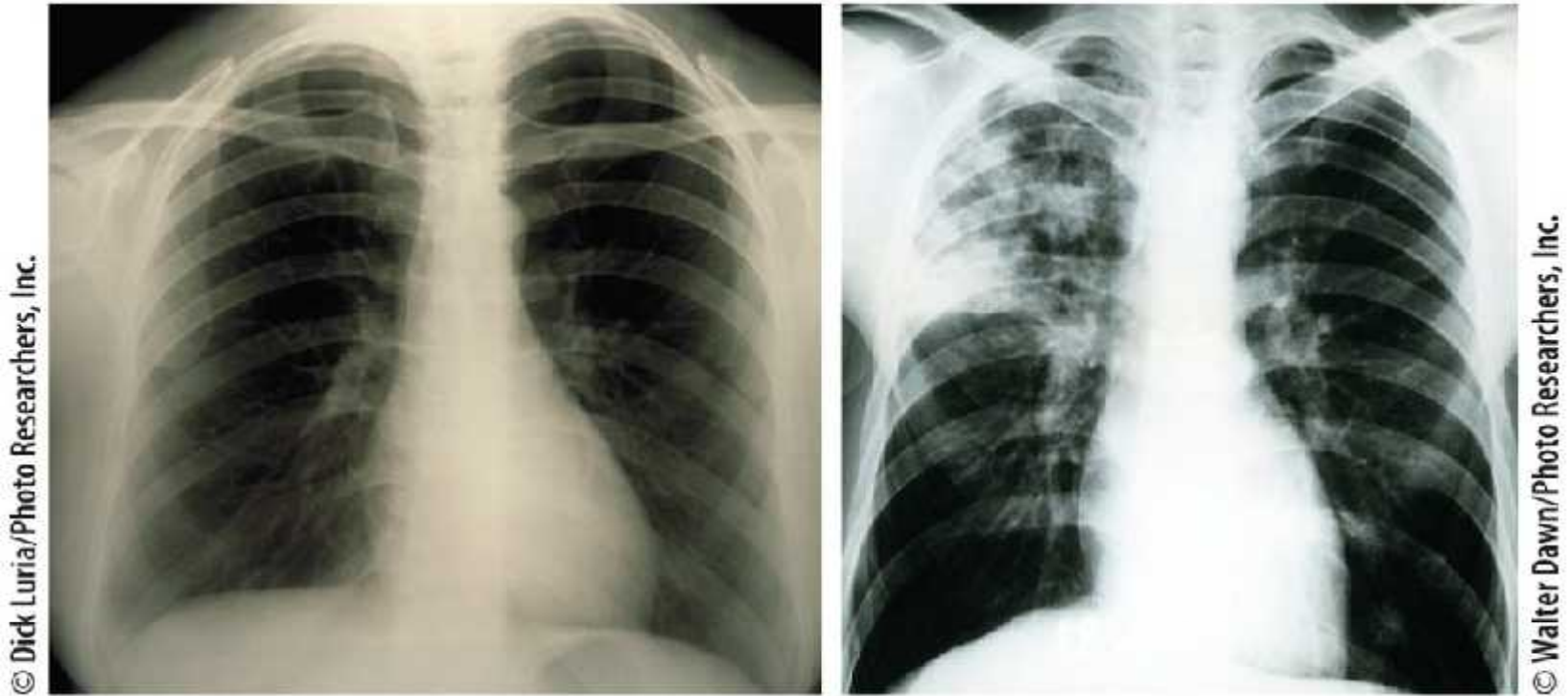
- **Pathogen study 2: *Mycobacterium tuberculosis***
  - Overcoming host defenses
    - Elimination of the microbe is mediated by T_H1 responses and upregulation of macrophage killing functions.

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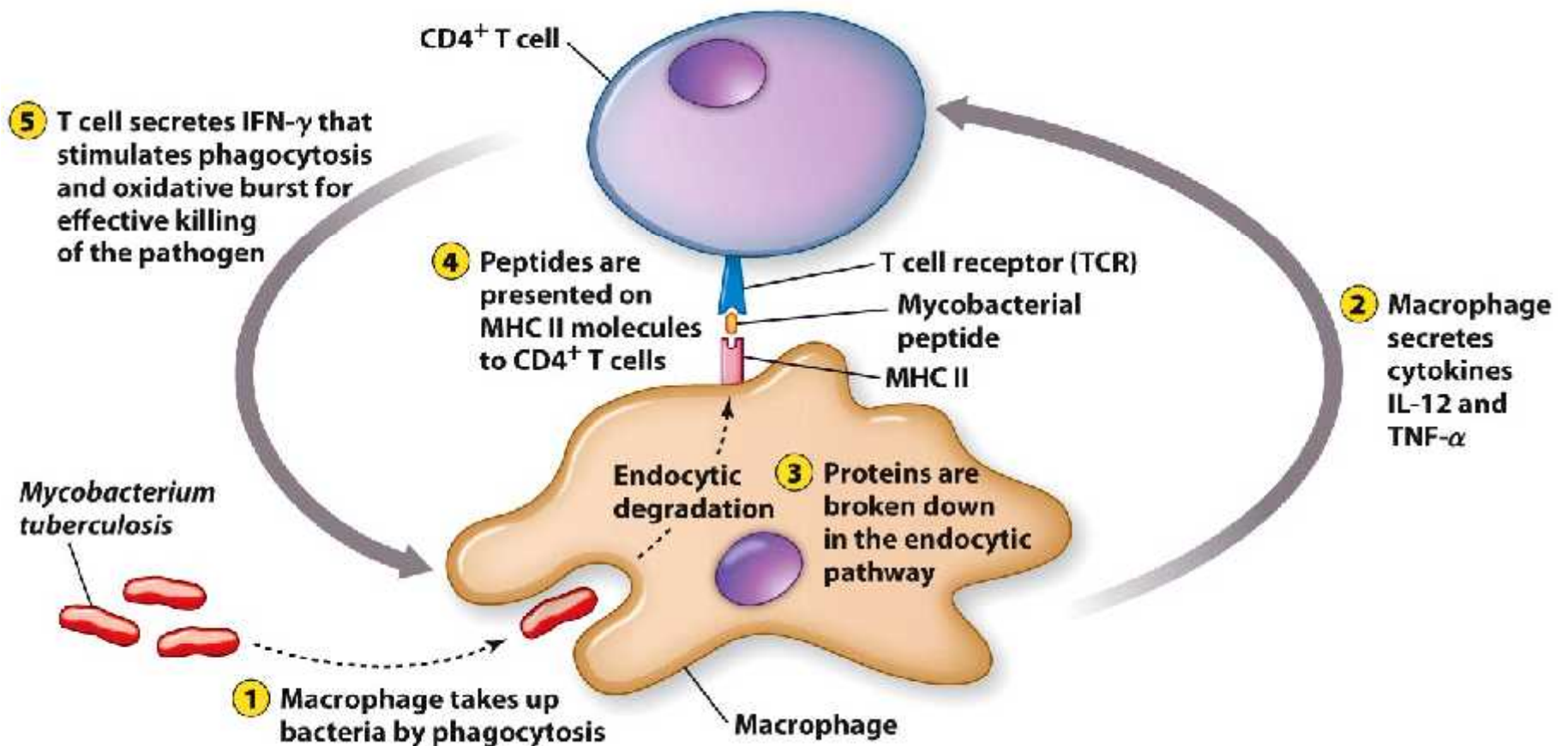
- **Pathogen study 2: *Mycobacterium tuberculosis***
  - Overcoming host defenses

    - Preventing phagosome fusion with lysosomes keeps bacteria alive in lung macrophages.

    - Bacteria also produce and surround themselves with lipoarabinomannan (LAM).
      - Downregulates oxidative burst
      - Neutralizes many toxic oxygen species

    - Superoxide dismutase and catalase can also be produced by the bacteria to protect the cells from oxidative damage.

X-rays of normal and diseased lungs

- **Pathogen study 2: *Mycobacterium tuberculosis***
  - Disease consequences
    - When the macrophages can't eliminate the microbe, chronic elevated TNF-α levels occur.
    - Chronic inflammation results in walled-off granulomas, damaging lung tissue.

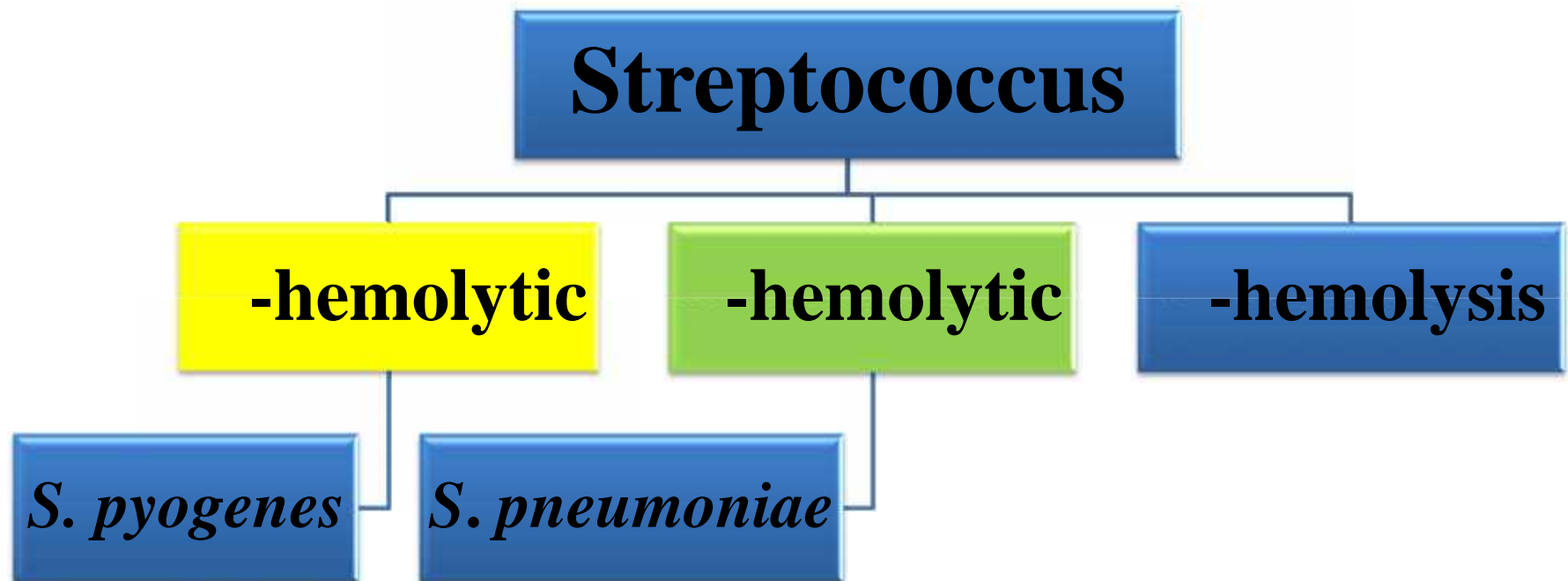# Bacteria as Human Pathogens : Examples

Staphylococcus

- ✓ **Staphylococci** are small spherical cells (1 μm) found in grapelike clusters. Staphylococci are nonmotile, catalase-producing bacteria. The genus Staphylococcus includes over 30 species and subspecies.

- ✓ *S. aureus* (and *E. coli*) are among the most frequent causal organisms in human bacterial infections.

- ●
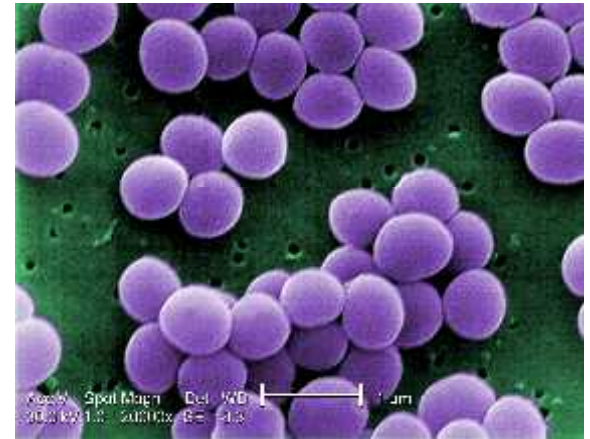
- *S. aureus* is frequently the causal pathogen in nosocomial infections. Certain strains are known to cause hospital epidemics.
- The most important preventive measure in hospitals is washing the hands thoroughly before medical and nursing procedures

Streptococcus

✓ **Streptococci** are Gram-positive, nonmotile, catalase-negative, facultatively anaerobic cocci that occur in chains or pairs. They are classified based on their hemolytic capacity (α, β, γ hemolysis) and the antigenicity of a carbohydrate occurring in their cell walls (**Lancefield antigen**)..

Streptococcus

-hemolytic

-hemolytic

-hemolysis

S. pyogenes

S. pneumoniae

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AGM MicrobeLibrary.org © Buxton

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- ✓ **β -hemolytic group** A streptococci (***S. pyogenes***) cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue

- ✓ The **α-hemolytic** pneumococci (***S. pneumoniae***) cause infections of the respiratory tract. Penicillins are the antibiotics of choice. Resistance to penicillins is known among pneumococci, and is increasing.

✓ Certain oral streptococci are responsible for **dental caries**.

✓ Oral streptococci also cause half of all cases of endocarditis. (en-do-kor-dy-tis)

✓ Although enterococci show only low levels of pathogenicity, they frequently cause nosocomial infections in immunocompromised patients (usually as elements of a mixed flora).

- **Enterococci**

✓ Are a widespread bacterial genus normally found in the intestines of humans and other animals.

✓ They are nonmotile, catalase-negative, and characterized by group antigen D. They are able to proliferate at 45 C, in the presence of 6.5% NaCl and at pH 9, qualities that differentiate them from streptococci. As classic opportunists, enterococci show only low levels of pathogenicity.

✓ they are frequently isolated as components of a mixed flora in nosocomial infections . Ninety percent of such isolates are identified as *E. faecalis*, 5–10% as *E. faecium*

✓ **Enterococci** frequently develop resistance to antibiotics. Strains manifesting multiple resistance are found mainly in hospitals, in keeping with the classic opportunistic.

Bacillus

- The genera **Bacillus** and **Clostridium** belong to the Bacillaceae family of sporing bacteria. There are numerous species in the genus Bacillus (e.g., ***B. cereus, B. subtilis***, etc.) that normally live in the soil. The organism in the group that is of veterinary and human medical interest is ***Bacillus anthracis***

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✓ **Gas gangrene** (*clostridial myonecrosis*). An aggressive infection of the musculature with myonecrosis and toxemia *(presence of toxins in the blood).* The incubation period varies from hours to a few days.

- **Therapy**. Primary treatment is surgical, accompanied by antibiosis (penicillins, cephalosporins). Treatment with hyperbaric *(use of oxygen at a level higher than atmospheric pressure)*

- **Epidemiology and prevention**. True gas gangrene is now a rare condition. Timely operation of contaminated wounds is the main preventive measure.

✓ *Clostridium tetani* **(Tetanus)**

- **Tetanus (lockjaw)** is an acute clostridial disease, its clinical manifestations do not result directly from the invasive infection, but are rather caused by a strong neurotoxin.
- **Pathogenesis and clinical picture**. These ubiquitous pathogens invade tissues following injuries . Given anaerobic conditions, they proliferate and produce the toxin, reaches the anterior horns of the spinal cord or brain stem

✓ *Clostridium botulinum* (Botulism)
- **Foodborne botulism** is not an infection, but rather an intoxication, that is, the toxin is ingested with food. Infant botulism involves ingestion of spores and wound botulism results from infection of a wound.
- **Toxin**. The very strong botulinum neurotoxin is a heat-labile protein. Seven toxigenic types are differentiated, each of which produces an immunologically distinct form of botulinum toxin. Types A, B, and E cause poisoning in humans.

- **Wound botulism** results from wound infection *by C. botulinum* and is very rare.

- **Infant botulism**, first described in 1976, results from ingestion of spores with food (e.g., honey). Probably due to the conditions prevailing in the intestines of infants up to the age of six months, the spores are able to proliferate there and produce the toxin. The lethality of infant botulism is low (<1%).

Enterobacteriaceae

– The most important bacterial family in human medicine is the Enterobacteriaceae.

– This family includes genera and species that cause well-defined diseases with typical clinical symptoms (typhoid fever, dysentery, plague) as well as many opportunists that cause mainly nosocomial infections (urinary tract infections, pneumonias, wound infections, sepsis).

– Enterobacteriaceae are Gram-negative, usually motile, facultatively anaerobic rod bacteria

- Their natural habitat is the intestinal tract of humans and animals.

- Some species cause characteristic diseases.

- While others are facultatively pathogenic, they are still among the bacteria most frequently isolated as pathogens (e.g., *E. coli*). They are often responsible for nosocomial diseases

Salmonella

Enteric salmonelloses

– develop when pathogens are taken up with food. The primary infection source is usually livestock.

– These relatively frequent infections remain restricted to the gastrointestinal tract.

– Treatment with anti infective agents is necessary in exceptional cases only

Shigella

Pathogenesis.

- Shigellae are only pathogenic in humans.

- The pathogens are ingested orally. Only a few hundred bacteria suffice for an infective dose.

- Shigellae enter the terminal ileum and colon, where they are taken up by the M cells in the intestinal mucosa, which in turn are in close vicinity to the macrophages.

- Following phagocytosis by the macrophages, the shigellae lyse the phagosome and actively induce macrophage apoptosis.

- The shigellae released from the dead macrophages are then taken up by enterocytes (**intestinal absorptive cells)**

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Shigella dysenteriae produces shigatoxin, which occur in several other Enterobacteriaceae.

The toxin inhibits protein synthesis in eukaryotic cells by splitting the 23S rRNA at a certain locus.

Shigatoxin contributes to the colonic epithelial damage, the small intestine diarrhea with watery stools

Escherichia coli
General characteristics

– The natural habitat of *E. coli* is the intestines of animals and humans. This bacterium is therefore used as an indicator for fecal contamination of drinking water, bathing water, and foods. Guideline regulations: 100 ml of drinking water must not contain any *E. coli.*

Morphology, culture, and antigen structure.

- The Gram-negative, straight rods are peritrichously flagellated.
- Lactose is broken down rapidly. The complex antigen structure of these bacteria is based on O, K, and H antigens. Specific numbers have been assigned to the antigens, e.g., serovar O18:K1:H7.

Vibrio cholerae

are Gram-negative rod bacteria, usually slightly bent (comma-shaped), 1.5–2 μm in length, and 0.3–0.5 μm wide, flagellated.

Culturing of *V. cholerae* is possible on simple nutrient mediums at 37 C in a normal atmosphere. Owing to its pronounced alkali stability, *V. cholerae* can be selectively cultured out of bacterial mixtures at pH 9.

Cholera toxin. Cholera toxin is the sole cause of the clinical disease. This substance induces the enterocytes to increase secretion of electrolytes, above all Cl– ions, whereby passive water loss also occurs.

Haemophilus influenza

- Hemophilic bacteria are so designated because they require growth factors contained in blood.

- The most important human pathogen in this genus is **H. influenzae**.

- Other Haemophilus species either infect only animals or are found in the normal human mucosal flora**.**

- **Haemophilus** are small (length: 1.0–1.5 µm, width: 0.3 µm), often encapsulated, nonmotile, Gram-negative rods.

- *H. influenzae* is a facultative anaerobe requiring growth factors X and V in its culture medium.

- The X factor is hemin, required by the bacteria to synthesize enzymes containing heme (cytochromes, catalase, oxidases).

- The V factor was identified as NAD or NADP.

- A standard blood agar plate does not contain sufficient free V factor.

- Some bacteria, in particular *Staphylococcus aureus*, produce excess NAD and even secrete this coenzyme into the medium.

- That is why *H. influenzae* can proliferate in the immediate vicinity of *S. aureus* colonies.

- This is known as the **satellite phenomenon**. The medium normally used to culture *H. influenzae* is chocolate agar containing sufficient amounts of the X and V factors.

Helicobacter pylori

Morphology and culture.

- *H. pylori* are spirally shaped, Gram-negative rods with lophotrichous flagellation.

- Cultures from stomach biopsies are grown on enriched mediums and selective mediums under microaerobic conditions (90% N_2, 5% CO_2, and 5% O_2) for three to four days.

- Identification is based on detection of oxidase, catalase, and urease

- *H. pylori* occurs only in humans and is transmitted by the fecal-oral pathway.
- The pathogen colonizes and infects the stomach mucosa.
- The pathogenicity factors include **pronounced motility** for efficient target cell searching, **adhesion** to the surface epithelial cells of the stomach, **urease** that releases ammonia from urea to facilitate survival of the cells in a highly acidic environment and a **vacuolizing cytotoxin** (VacA) that destroys epithelial cells.

Pseudomonas aeruginosa

- **Occurrence**, **significance**.
- All pseudomonads are widespread in nature.
- They are regularly found in soils, surface water, including the ocean, on plants and, in small numbers, in human and animal intestines.
- They can proliferate in a moist environment containing only traces of nutrient substances.
- The most important species in this group from a medical point of view is *P. aeruginosa*, which causes infections in person with immune defects

Morphology and culture.

 P. aeruginosa are plump, 2–4 µm long rods with one to several polar flagella. G-

- Some strains can produce a viscous extracellular slime layer. These mucoid strains are frequently isolated in material from cystic fibrosis patients.

- *P. aeruginosa* possesses an outer membrane as part of its cell wall. The architecture of this membrane is responsible for the natural resistance of this bacterium to many antibiotics.

Mycoplasmas

- are bacteria that do not possess rigid cell walls for lack of a murein layer. These bacteria take on many different forms. They can only be rendered visible in their native state with phase contrast or dark field microscopy.

- *M. pneumoniae* frequently causes pneumonias that run atypical courses, especially in youths. Ten to twenty percent of pneumonias contracted outside of hospitals are caused by this pathogen

Nosocomial Infections

- The term nosocomial infection designates infections contracted by hospitalized patients 48 hours or more from the beginning of hospitalization. These are secondary infections that occur as complications of the primary diseases to be treated in the hospital

- Nosocomial infections occur in hospitalized patients as complications of their primary disease. Such infections are reported in an average of approximately 3.5% (Germany) to 5% (USA) of all hospitalized patients, in tertiary care hospitals in about 10% and in the intensive care units of those in about 15–20% of cases. The most frequent types of infection are urinary tract infections (42 %), pneumonia (21%), surgical wound infections (16%), and sepsis (8 %).

Pathogens, Infections, Frequency

- The significance of the different human pathogens in nosocomial infections varies widely:

- **Subcellular entities.** Isolated cases of Creutzfeldt-Jakob disease due to unsterilized instruments have been described in the literature. Such accidents now no longer occur.

- There are no reliable figures available on viral nosocomial infections. A rough estimate puts viral nosocomial infections at less than 1% of the total. An example of a viral nosocomial infection is infectious hepatitis transmitted by blood or blood products

- **Bacteria** are the main pathogens involved in nosocomial infections. Most of the causative organisms are facultatively pathogenic (opportunistic) bacteria, which are frequently resistant to many different antibiotics. These bacteria have found niches in which they persist as so-called hospital flora. The resistance patters seen in these bacteria reflect the often wide variations between anti-infective regimens as practiced in different hospitals.

Fungi. Fungal nosocomial infections have been on the increase in recent years. It can be said in general that they affect immunocompromised patients and that neutropenic patients are particular susceptible

Microbiology for Nursing students

Chapter Six: Control of Infectious Disease
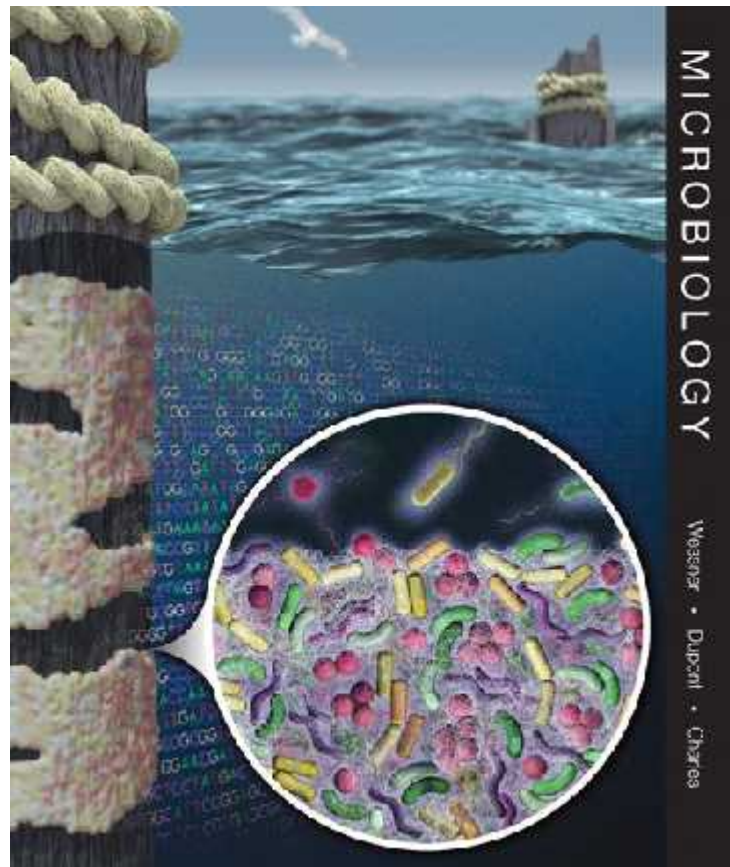
Dr. Sulaiman Alnaimat 2015

Introduction:

- Human control of disease has made incredible progress in the last century.
  - Due to vaccines, antimicrobial drugs, and improvements in sanitation and hygiene
  - In United States, premature death from infectious disease has dropped considerably.
- This chapter will examine these methods and new challenges that could reverse the improvements.
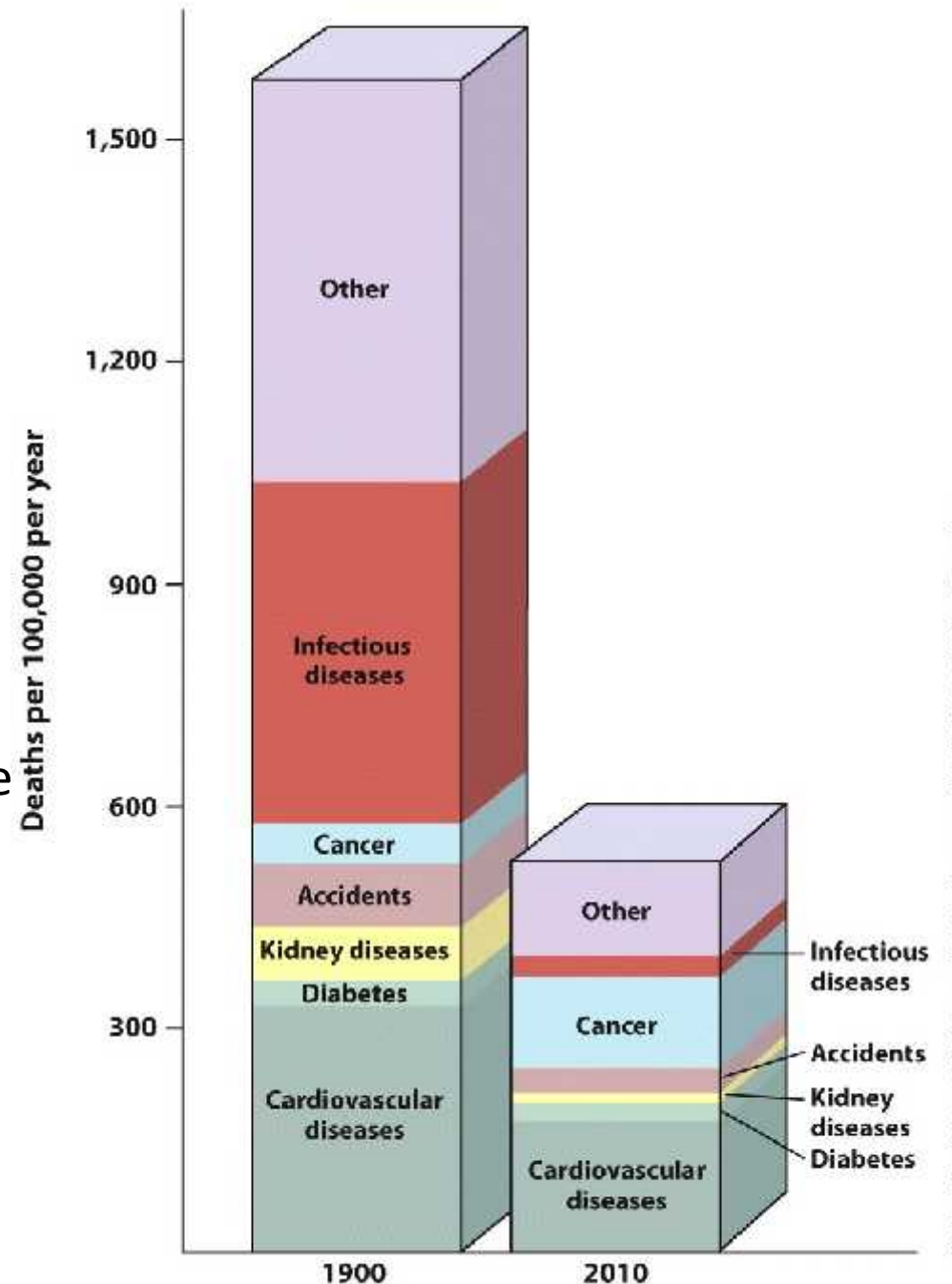
Eliminating microbes and preventing their growth:

- *How can we eliminate microbes or inhibit their growth?*
  - Just as we need ways to GROW microbes for further study, we also need ways to PREVENT their growth or to remove them from some locations....

Eliminating microbes and preventing their growth:

- Physical removal of microbes by filtration
  - Filtration has been a method of purifying liquids for centuries (using sand, charcoal, etc.).
  - Newer methods use nylon/Teflon filters with a pore size of 0.2 or 0.45 μm (small enough to keep out most eukaryal and bacterial cells).
  - Viruses can be removed from liquids by ultrafiltration methods (reducing pore size 10 to 100 nm).
- Problems can result, though…
  - Large particles clog filters.
  - Ultrafiltration requires high pressure.
  - Viscous liquids don't filter well.

LifeStraw®

- Water filters convert
contaminated water into clean,
safe drinking water. The easy-
to-use filters are a vital tool for
some of the 780 million people
who don't have ready access to
safe drinking water. This
leaves them at risk for diarrheal
disease, which kills more than
1.5 million people every year.
Safe drinking water is especially
important for vulnerable
groups, such as children under
five, pregnant women and
people living with HIV.

LifeStraw

is a water filter designed to be used by one person to filter water so that they may safely drink it. It filters a maximum of 1000 litres of water, enough for one person for one year. It removes almost all of waterborne bacteria and parasites.

- Temperature manipulation: Heat
  - Heat denatures proteins and nucleic acids.
  - Heating to 100°C kills most microbes quickly.
  - An autoclave adds pressure, keeping fluids from evaporating during the high temperatures, BUT
    - Some microbes thrive in higher heat (hyperthermophiles).
    - Some microbial structures resist high temps (endospores).
    - Some materials can't be heated.

Health Protection Agency/Photo Researchers, Inc.

Eliminating microbes and preventing their growth:

- Temperature manipulation—lower heat
  - Pasteurization (low-temp heating) to reduce microbe numbers
- Temperature manipulation—freezing
  - Can damage cells by forming ice crystals
  - Can stop biochemical reactions in microbes
  - Good for long-term preservation

Courtesy Christine Dupont

Eliminating microbes and preventing their growth:

- Using electromagnetic radiation to control microbes
  - UV radiation of 260 to 280 nm wavelengths can damage DNA, forming thymine dimers.
  - This can be exploited to control microbial growth on non-living surfaces and in water.

9

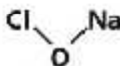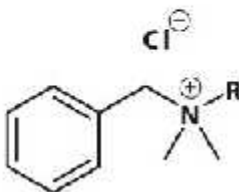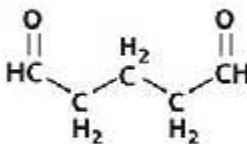# Eliminating microbes and preventing their growth:
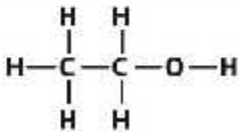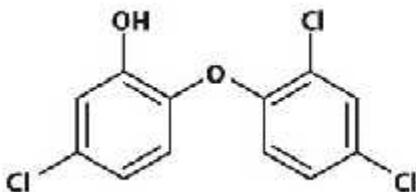
- Chemical methods of controlling microbes
  - Many chemicals can kill microbes, or inhibit their growth.
  - Disinfectants: Chemicals used on non-living surfaces to kill potentially infectious microbes.
  - Antiseptics: Chemicals that can be used on living tissue to kill potentially infectious microbes (usually only used topically)

Eliminating microbes and preventing their growth:

- Chemical methods of controlling microbes
  - What makes a chemical a "good" microbe-killer?
    - Should kill a wide-range of microbes
    - Shouldn't be corrosive or overly toxic
    - Shouldn't leave a residue
    - Shouldn't emit fumes or smell TOO bad
    - Should be cheap
    - Should be temperature stable

- Chemical methods of controlling microbes

TABLE 6.5 Commonly used disinfectants

Class	Example	Structure	Notes
Alcohols	Ethanol		Routinely used in laboratory settings; also present in most hand sanitizers
Phenolic compounds	Triclosan		Added to numerous products, including some soaps, deodorants, and cosmetics
Oxidizing agents	Sodium hypochlorite		Commonly added to swimming pools and hot tubs to inhibit microbial growth
Others	Benzalkonium chloride	$R = C_8H_{17} - C_{18}H_{37}$	Major ingredient in Lysol®
	Glutaraldehyde		Often used to prepare biological specimens

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Eliminating microbes and preventing their growth:

- Practical issues for destroying microbes or preventing their growth
  - How do you pick a method?
    - What microbes are present? What about endospores?
    - How many microbes are present? Do they all need to die?
    - What kind of object needs to be treated?
    - If using a physical method, how long/intense does it need to be?
    - If using a chemical method, how powerful must it be, and how long does it need to be applied?
    - Do we need to worry about toxicity to humans or other life?
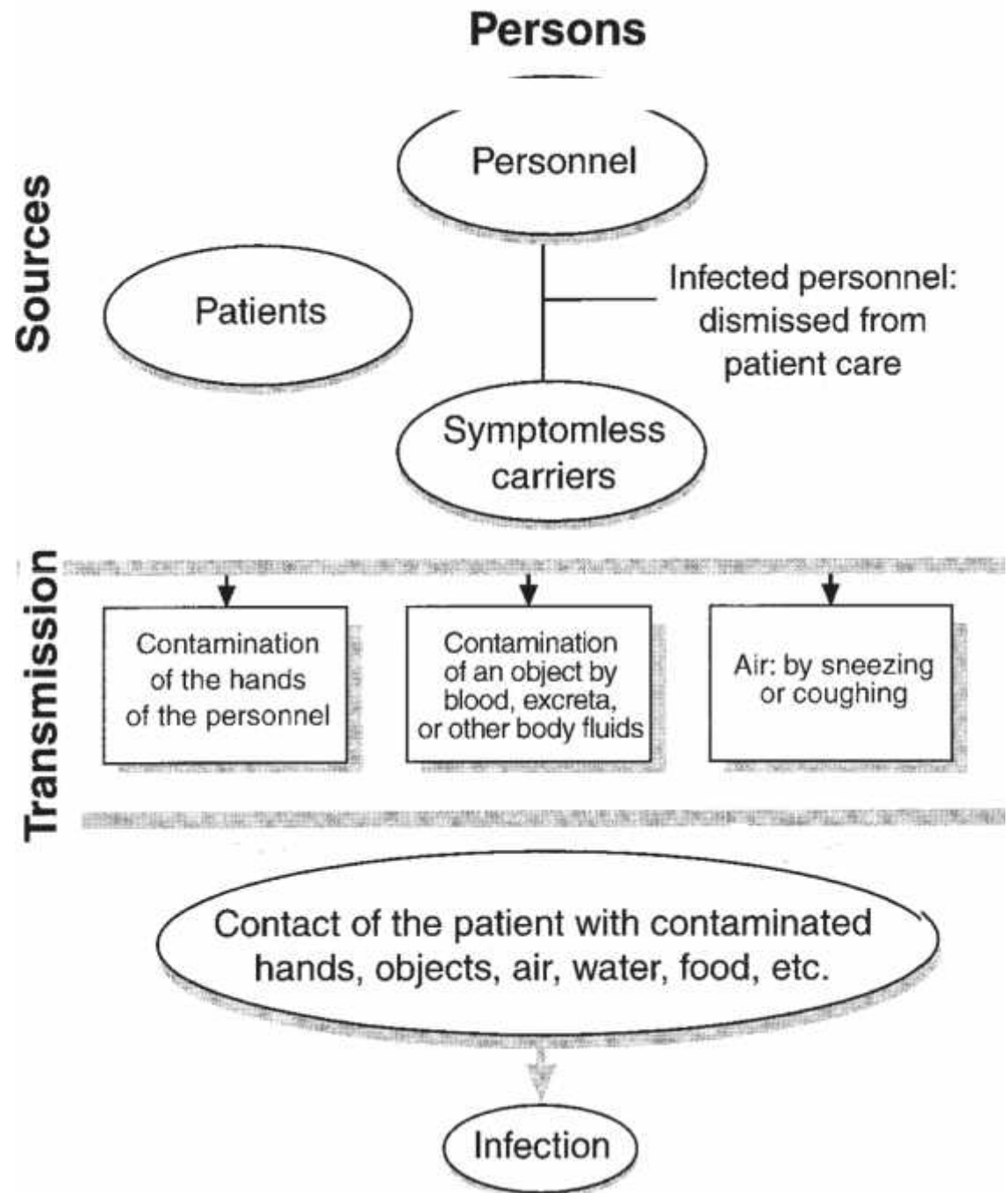
Hospital hygiene and infection control

- Nosocomial infections:
  - Known also as hospital-acquired infections, hospital-associated infections, and hospital infections
  - Are infections that are not present in the patient at the time of admission to hospital but develop during the course of the stay in hospital

14

Safety, WHO Patient, and World Health Organization. "WHO guidelines on hand hygiene in health care." (2009).
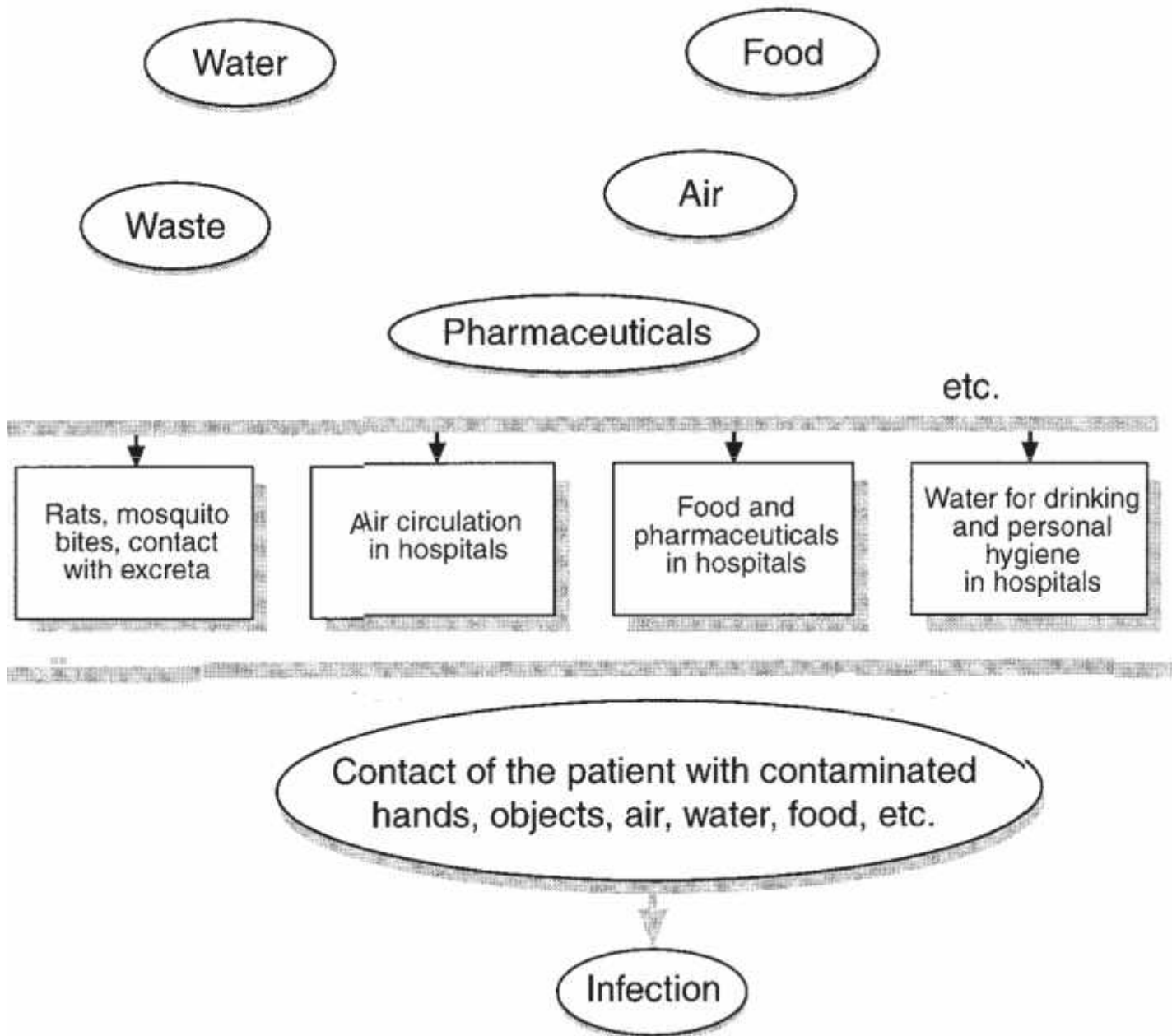
The prevention of nosocomial infection

- Principles
  - Two basic principles govern the main measures that should be taken
- in order to prevent the spread of nosocomial infections in health-care facilities:
  - separate the infection source from the rest of the hospital;
  - cut off any route of transmission.

Safety, WHO Patient, and World Health Organization. "WHO guidelines on hand hygiene in health care." (2009). 15

All objects that come in contact with patients should be considered as potentially contaminated

16

Environment

Water

Food

Air

Waste

Pharmaceuticals

etc.

| Rats, mosquito bites, contact with excreta | Air circulation in hospitals | Food and pharmaceuticals in hospitals | Water for drinking and personal hygiene in hospitals |

Contact of the patient with contaminated hands, objects, air, water, food, etc.

Infection

The prevention of nosocomial infection

1. Isolation of infected patients and standard precautions
   - The first essential measure in preventing the spread of nosocomial infections is *isolation of infected patients.*

2. Cleaning
   - One of the most basic measures for the maintenance of hygiene, and one that is particularly important in the hospital environment, is cleaning.
   - The principal aim of cleaning is to remove visible dirt. It is essentially a mechanical process: the dirt is dissolved by water, diluted until it is no longer visible, and rinsed off. Soaps and detergents act as solubility promoting agents

Safety, WHO Patient, and World Health Organization. "WHO guidelines on hand hygiene in health care." (2009). 18

The prevention of nosocomial infection

3. *Sterilization*
    – Self-evidently, an object should be sterile, i.e. free of microorganisms, after sterilization.
    – Sterilization can be achieved by both physical and chemical means. Physical methods are based on the action of heat (autoclaving, dry thermal or wet thermal sterilization), on irradiation (g-irradiation), or on mechanical separation by filtaration . Chemical means include gas sterilization with ethylene oxide or other gases.

Safety, WHO Patient, and World Health Organization. "WHO guidelines on hand hygiene in health care." (2009).

The prevention of nosocomial infection

4. Hand hygiene
   - As the hands of health-care workers are the most frequent vehicle of nosocomial infections, hand hygiene-including both hand washing and hand disinfection-is the primary preventive measure.

   - Killing all transient flora with all contaminants within a short time (a few seconds) necessitates hygienic hand disinfection: only alcohol or alcoholic preparations act sufficiently fast. Hands should be disinfected with alcohol when an infected tissue or body fluid is touched without gloves.

Safety, WHO Patient, and World Health Organization. "WHO guidelines on hand hygiene in health care." (2009).

Box 14.2 Essentials of the standard precautions to be used in the care of all patients

A. **Hand washing**
- Wash hands after touching blood, secretions, excretions and contaminated items, whether or not gloves are worn. Wash hands immediately after gloves are removed, between patient contacts.
- Use a plain soap for routine hand washing.
- Use an antimicrobial agent for specific circumstances.

B. **Gloves**
- Wear gloves when touching blood, body fluids, secretions, excretions, and contaminated items. Put on clean gloves just before touching mucous membranes and non-intact skin.

Safety, WHO Patient, and World Health Organization. "WHO guidelines on hand hygiene in health care." (2009).

Antimicrobial drugs:

- *What kinds of drugs are used to treat infections and how do they work?*
  - Antimicrobial drugs are different from antiseptics and disinfectants.
    - They are often administered internally.
    - They exhibit selective toxicity (more toxic to an infectious microbe than to the host/host cells).
    - They may be of natural origin or chemically synthesized.
    - They may be highly effective at eliminating one class of microbes (e.g., bacteria), while ineffective at eliminating others.
    - Their history of use extends across the last century.

Streptomycin is introduced. One year later, resistance is reported for *Mycobacterium tuberculosis.*
1947

Penicillin is mass-produced.
1943
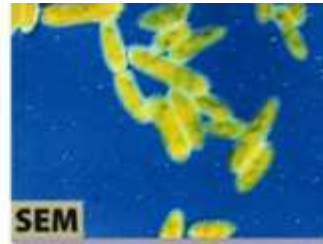
Tetracycline resistance appears in *Shigella dysenteriae.*
1956

Methicillin is introduced.
1959

Cephalothin of the new cephalosporin group is introduced.
1964

1930
The first successful therapeutic use of **penicillin** is reported.

1945
Penicillin use begins worldwide. Within a few years, 20% of *Staphylococcus aureus* hospital isolates are penicillin resistant.

1952
Tetracycline is introduced.

1958
Vancomycin is introduced but rarely used until the mid-1980s.

1961
Methicillin-resistant *S. aureus* (MRSA) appears.

1966
Cephalothin resistance appears in several Gram-negative species.
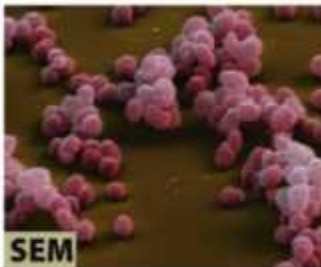
Gentamicin is introduced.
1967

Transferable **penicillinase** appears in *Neiserria gonorrhoeae.*
1976

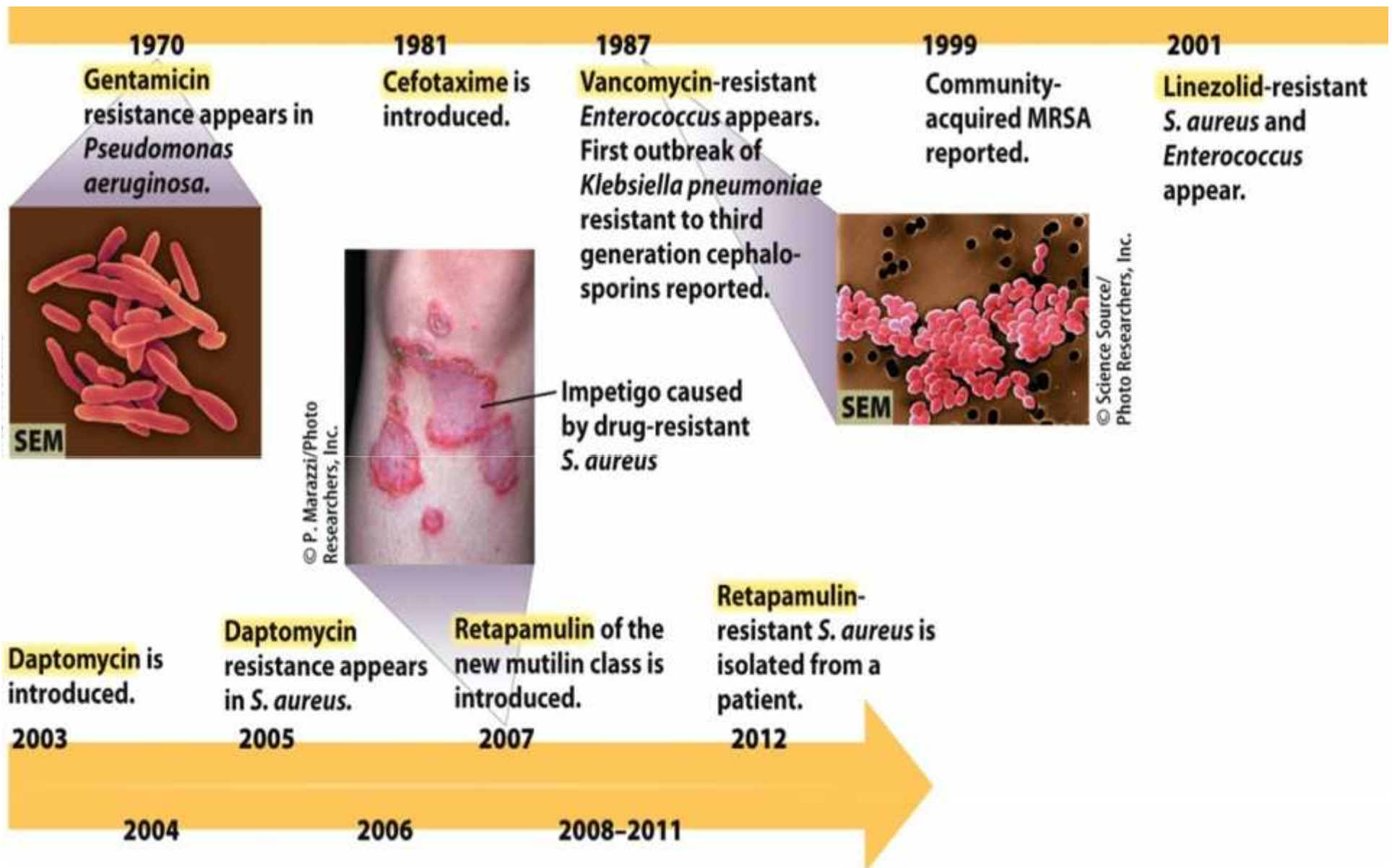**Cefotaxime** resistance appears. First penicillin-resistant *Enterococcus* appears.
1983

Intermediate resistance to **vancomycin** appears in *S. aureus.*
1996
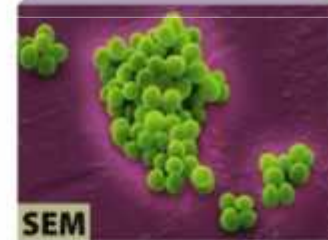
Linezolid of the new oxazolidinone class is introduced.
2000

Complete resistance to **vancomycin** appears in *S. aureus.*
2002

23

1970
Gentamicin resistance appears in *Pseudomonas aeruginosa.*

SEM

1981
Cefotaxime is introduced.

© P. Marazzi/Photo Researchers, Inc.

Impetigo caused by drug-resistant *S. aureus*

1987
Vancomycin-resistant *Enterococcus* appears. First outbreak of *Klebsiella pneumoniae* resistant to third generation cephalo-sporins reported.

1999
Community-acquired MRSA reported.

SEM

© Science Source/ Photo Researchers, Inc.

2001
Linezolid-resistant *S. aureus* and *Enterococcus* appear.

Daptomycin is introduced.
2003

Daptomycin resistance appears in *S. aureus.*
2005

Retapamulin of the new mutilin class is introduced.
2007

Retapamulin-resistant *S. aureus* is isolated from a patient.
2012

2004

2006

2008–2011

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Antimicrobial drugs:

- Antibiotics are the most important, historically.
  - Alexander Fleming in 1928
    - Observations of bread mold *Penicillium* inhibiting *S. aureus* cultures
  - Led to a surge in research on naturally occurring antibiotic compounds
  - Analysis of molecular structure
    - Led to classification
    - Further research on devising synthetic equivalents

- Antibiotics often interfere with
  - Peptidoglycan synthesis
  - Membrane integrity
  - DNA synthesis
  - Transcription
  - Folic acid synthesis
  - Ribosome function

- Structure often determines what can be affected.
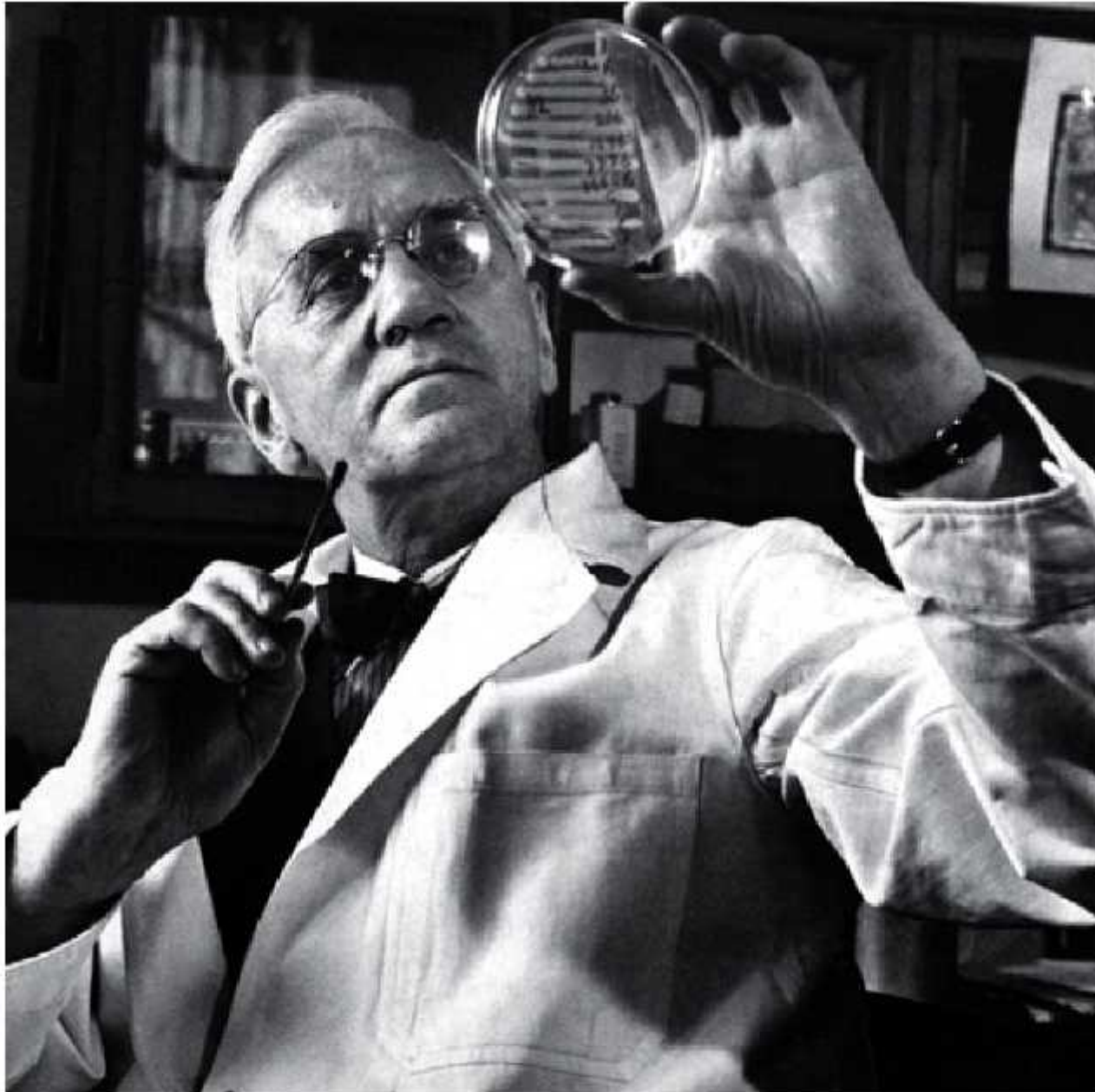
Figure 27.1c Microbiology: An Evolving Science
Science Source/Photo Researchers, Inc.

Figure 31.27

Staphylococcus Penicillium

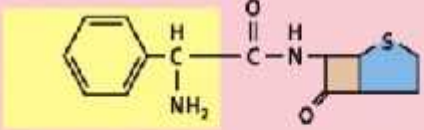Zone of inhibited growth

© 2011 Pearson Education, Inc.
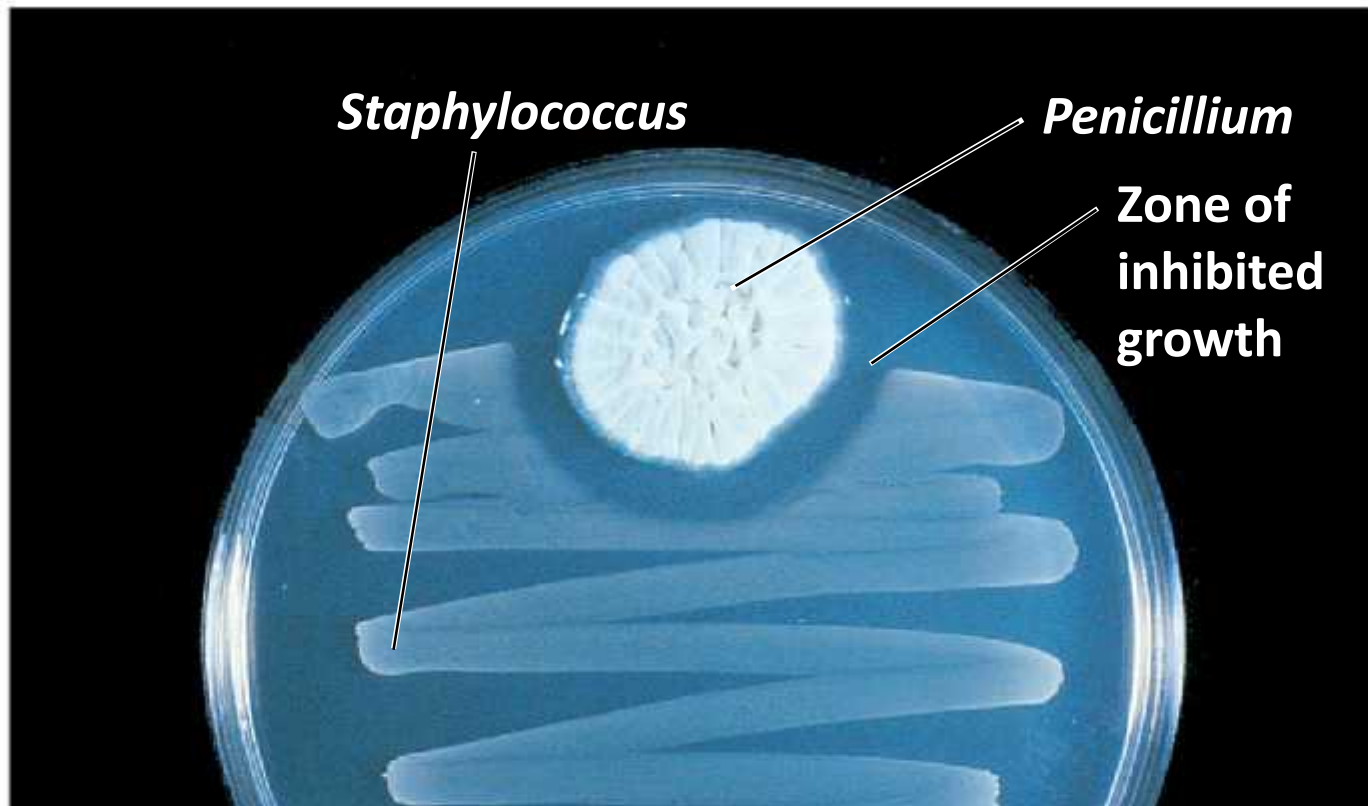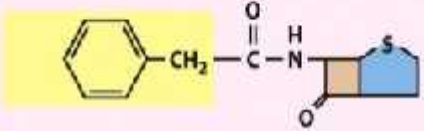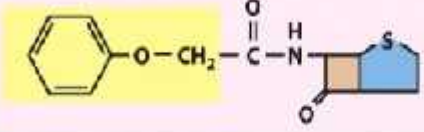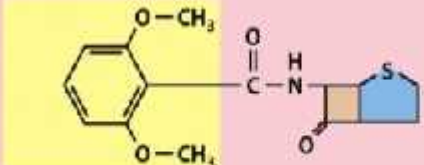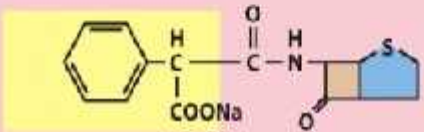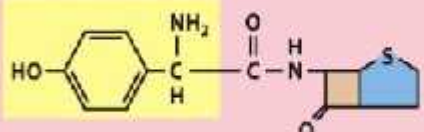
TABLE 24.1 Antibiotics and their origins

Source	Natural antibiotic	Semisynthetic derivatives
Antibacterial antibiotics		
Penicillium chryosgenum (fungi)	Penicillin G, penicillin V	Methicillin, ampicillin, amoxicillin, carbenicillin, oxacillin
Cephalosporium species (fungi)	Cephalosporin	Cephalexin, cephradine, cefradoxil, ceftazidime
Streptomyces griseus	Streptomycin	NA[a]
Streptomyces aureofaciens	Tetracycline	Doxycycline, oxytetracycline
Streptomyces venezuelae	Chloramphenicol	NA[a]
Streptomyces erythreus	Erythromycin	Azithromycin, clarithromycin
Streptomyces kanamyceticus	Kanamycin	Amikacin, arbekacin
Streptomyces tenebrarius	Tobramycin	NA[a]
Streptomyces fradiae	Neomycin	NA[a]
Streptomyces mediterranei	Rifamycin	Rifampin
Amycolatopsis orientalis	Vancomycin	Ramoplanin
Micromonospora species	Gentamicin	NA[a]
Bacillus licheniformis	Bacitracin	NA[a]
Bacillus polymyxa	Polymyxins	NA[a]
Antifungal antibiotics		
Penicillium griseofulvum	Griseofulvin	NA[a]
Streptomyces nodosus	Polyenes	NA[a]

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[a]NA: not applicable. Not developed or not commonly used.

Structure	Name	Feature
Natural penicillins		
	Penicillin G	Active against Gram-positive bacteria
	Penicillin V	Active against Gram-positive bacteria and is acid resistant
Semisynthetic penicillins		
	Methicillin	β-lactamase resistant
	Ampicillin	Active against a broad spectrum of bacteria and is acid resistant
	Carbenicillin	Active against a broad spectrum of bacteria
	Amoxicillin	Active against a broad spectrum of bacteria

Structure of natural and semisynthetic penicillins

β-lactam ring

Penicillins   Cephalosporins
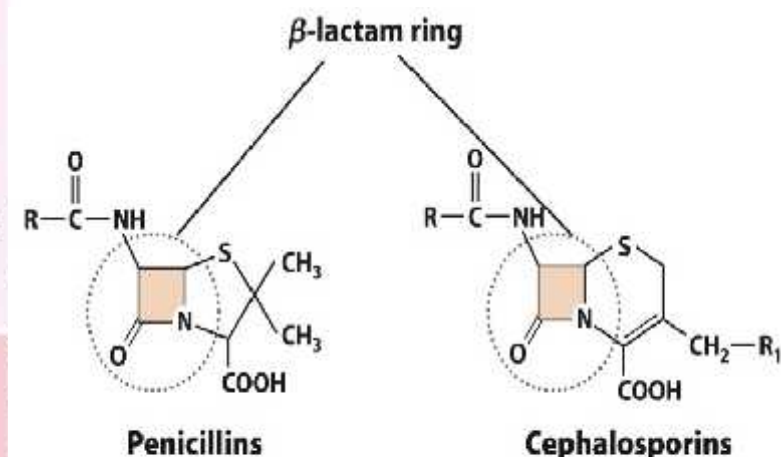
Difference between penicillins and cephalosporins

Black, *Microbiology: Principles and Explorations*, copyright 2012, John Wiley & Sons, Inc. This material is reproduced with permission of John Wiley & Sons, Inc.

TABLE 24.2 Action, structure, and targets of antibacterial drug groups
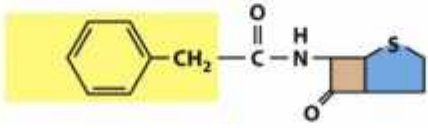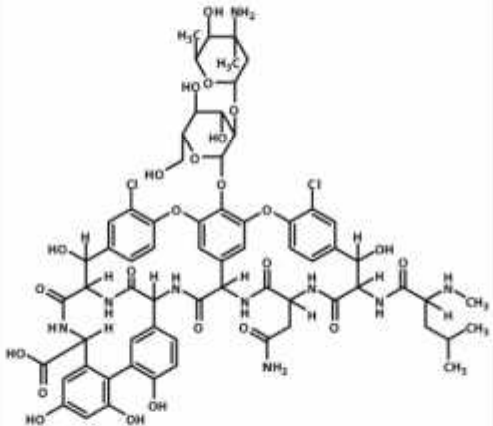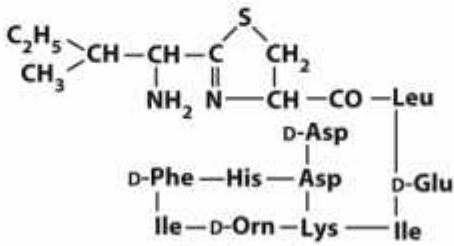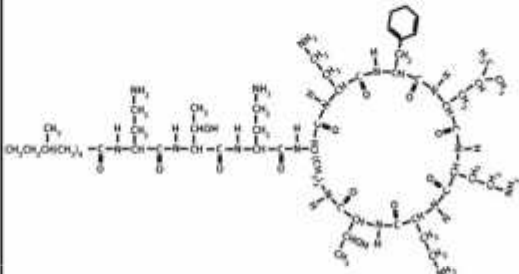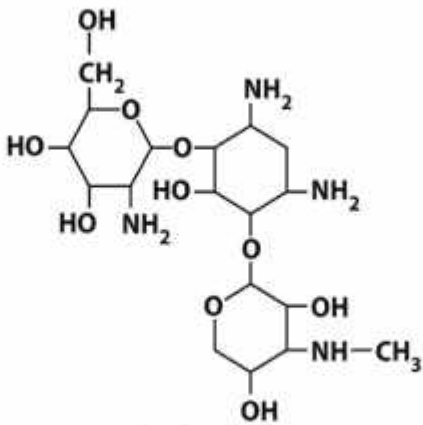
Action	Class/group	Structure	Target	Examples
Antibacterial drugs				
Antibiotics				
Inhibition of peptidoglycan synthesis	β-lactams	Penicillin G	Peptidoglycan transpeptidases (PBPs)	Penicillins G and V, methicillin, cephalosporins, monobactams, carbenicillin
	Glycopeptides	Vancomycin	Peptidoglycan peptide subunits	Vancomycin, avoparcin
	Bacitracin (topical use)	Bacitracin	Isoprenyl pyrophosphate	Bacitracin

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TABLE 24.2 Action, structure, and targets of antibacterial drug groups

Action	Class/group	Structure	Target	Examples
Antibacterial drugs				
	Antibiotics			
Disruption of membranes	Polymyxin B (topical use)	Polymyxin B	Membranes	Polymyxin B, polymyxin E
Inhibition of ribosome function	Aminoglycosides	Gentamicin	16S rRNA of 30S ribosome subunit	Gentamicin, neomycin, streptomycin, tobramycin

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TABLE 24.2 (Continued)

Action	Class/group	Structure	Target	Examples
	Macrolides	Erythromycin	Peptidyl transferase site of 50S ribosome subunit	Erythromycin, spectinomycin, carbomycin
	Tetracyclines	Tetracycline	30S ribosome subunit	Tetracycline, doxycycline, oxytetracycline
	Chloramphenicol	Chloramphenicol	23S rRNA of 50S ribosome subunit	Chloramphenicol
Inhibition of transcription	Rifamycins	Rifampin	β-subunit of bacterial RNA polymerase	Rifampin, rifabutin, rifapentine

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TABLE 24.2 (Continued)

Action	Class/group	Structure	Target	Examples
	Synthetic drugs			
Inhibition of nucleic acid synthesis	Quinolones	Ciprofloxacin	Gram-negative DNA gyrase or Gram-positive topoisomerase IV	Nalidixic acid, oxolinic acid, fluoroquinolones (e.g., ciprofloxacin)
	Sulfonamides	Sulfanilamide	Dihydropteroate synthetase (folic acid pathway)	Sulfisoxazole, sulfanilamide
	Trimethoprim	Trimethoprim	Dihydrofolate reductase (folic acid pathway)	Trimethoprim

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Antimicrobial drugs:

- Antibacterial drugs
  - Bacteriocidal = Directly kill the microbes treated
  - Bacteriostatic = Prevent replication of bacteria but do not kill them
  - Selected classes for further examination
    - Inhibitors of peptidoglycan synthesis
    - Inhibitors of ribosome function
    - Inhibitors of nucleic acid synthesis

Gram-positive

Plasma membrane

Gram-negative

Outer membrane

Cell membranes

Polymyxin B

PABA

Sulfonamides

Trimethoprim

Folic acid derivatives

Folic acid synthesis

Macrolides
Chloramphenicol

Ribosomes (protein synthesis)

50S

30S

Aminoglycosides
Tetracyclines

A, G   Nucleotides   T

+

DNA synthesis

DNA

RNA polymerase

mRNA

Transcription

Quinolones

Rifamycins

Peptidoglycan

Penicillin-binding proteins (PBPs)

Vancomycin
Bacitracin

Transport through outer membrane

β-lactams

Peptidoglycan synthesis

Based on Figure 11-1. Antimicrobial Chemotherapy.
Harold Neu and Thomas Gootz.

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Antifungal drugs

- Much more problematic (eukaryotes!)
- Very few available drugs , and the few must focus on
  - Disruption of cell ergosterol (instead of cholesterol in human cell membranes)
  - Inhibition of chitin cell wall structures
  - Selective inhibition of fungal mitosis (difficult!)
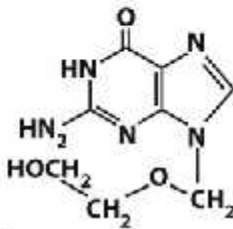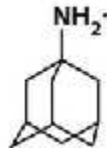
- ## Antiviral drugs

  - Tricky because viruses often use host cell processes
    - If you inhibit a host cell process, toxicity will be HIGH.
  - Common mechanisms involve
    - Inhibition of nucleic acid synthesis
      - Often through nucleoside/nucleotide analogues (AZT, acyclovir)
      - Inhibition of virus life cycle steps (intracellular uncoating of flu virus)

TABLE 24.3 Action, structure, and targets of antifungal drug groups

Action	Class/group	Structure	Target	Examples
Antifungal drugs				
Antibiotics				
Inhibition of mitosis	Griseofulvin	Griseofulvin	Tubulin	Griseofulvin
Disruption of plasma membrane	Polyenes	Amphotericin B	Plasma membrane ergosterol	Amphotericin B, nystatin
	Synthetic drugs			
	Azoles	Fuconazole	C14–demethylase	Fuconazole, ketoconazole, chlotrimazole, miconazole

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TABLE 24.5 Action, structure, and targets of antiviral drug groups

Action	Class/group	Structure	Target	Examples
Antiviral drugs				
Inhibition of DNA synthesis	Nucleoside analogs	Acyclovir (guanine analog)	DNA	Acyclovir, AZT, ribavirin
Inhibition of influenza virus uncoating	Amantadine	$NH_2 \cdot HCl$ Amantadine	Influenza virus M2 protein (an ion channel)	Amantadine, rimantadine

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Antimicrobial drug resistance:

- *How do microbes become drug-resistant?*
- Adaptation to selective pressures drives genetic change in microbes.
- Drug resistance genetic changes are negative.
    - The CDC estimates 2 million people contract bacterial infections in hospitals yearly.
    - Of these, approximately 90,000 die.
    - About 70% of the bacterial pathogens causing these infections show resistance to at least one antibacterial drug.

Antimicrobial drug resistance:

- So what is the evidence for selection for resistance in clinical settings?
  - Changes in antimicrobial drug use are positively correlated with changes in prevalence of resistance.
  - Increasing antibiotic treatment lengths increases resistant-microbe colonization rates.
  - The more antimicrobial drug use in a facility, the more drug resistance that can be found.
  - Patients with resistant strains receive antibiotics more often (providing selective pressure).

Antimicrobial drug resistance:

- Molecular mechanisms of resistance
  - Generally due to four possible mechanisms:
    1. Producing enzymes that modify or destroy the drug
    2. Altering binding targets of drugs
    3. Preventing drug entry into the target cell
    4. Pumping the drug back out of the target cell (efflux)

Drug inactivation uses enzymes that modify the drug.

Alteration of the drug target site prevents the drug from binding to its target.

Drug inaccessibility preventing entrance of the drug into the cell by modifying membrane permeability or transport systems.

Antibiotic

Antibiotic

R plasmid

Efflux pump

Antibiotic-altering enzyme

Drug efflux pumps the drug out of the cell using efflux pumps.

Antibiotic-degrading enzyme

Antibiotic

Antibiotic resistance genes

Antibiotic

Drug inactivation uses enzymes that degrade the drug.

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Antimicrobial drug resistance:

- The origin of drug resistance genes
  - Antimicrobial resistance in farmed animals
    - Agriculture is the 2nd largest consumer of antimicrobial drugs.
    - 40% of all antibiotic use is in farmed animals.
      - Antibiotic use increases animal size and weight.
      - Healthier animals = more profit
    - A difficult problem to define fully or change by policy as different countries have different standards and policies for agriculture and farm animal feeds.
    - It is entirely possible that overuse of antibiotics may contribute to antimicrobial resistance in human pathogens, but data is inconclusive to this point.

Antimicrobial drug resistance:

- Combating drug resistance
  - So what do we DO about this problem?
  - Several approaches exist that, if used collectively, may help reduce the problem.
    - Reduce use.
    - Use selective drugs.
    - Use multidrug cocktails.
    - Use effective infection control.
    - Develop new vaccines and improve access.
    - Develop alternatives/develop drugs in a smarter way.

    *Think about each of these approaches – How would you implement them? What are the benefits?

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Immunization and vaccines:

- *What are vaccines, and how are they used to control infectious disease?*
  - History of vaccination
    - Smallpox is a historic illness plaguing humankind.
      - It is viral in nature, and easily transmitted via aerosols.
      - The mortality rate is very high, ranging up to 50–90%.
    - Previous control attempts had been made but weren't very safe or as effective as necessary.
    - Edward Jenner coined the term "vaccination" in 1798 with his work protecting people from smallpox.
      - Jenner observed that milkmaids had smallpox less frequently than the rest of the population but they would get a lesser disease (cowpox).
      - He purposely infected a boy with cowpox, then later with smallpox—and the boy didn't develop the disease.

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Immunization and vaccines:

- Vaccine design
  - Vaccines confer protection by initiating immune memory.
    - Specialized T and B cells that are produced post-stimulation
  - The ideal vaccine generates a high level of immune memory without serious side effects.
  - Different types of vaccines include
    - Attenuated
    - Inactivated
    - Subunit
    - DNA

Immunization and vaccines:

- Vaccine design: Attenuated vaccines
  - Composed of living (but weakened) pathogen
  - Tend to produce HIGH immunity because the microbe replicates in the body
    - This exposes the immune system to a higher level of foreign antigen over a greater period of time.
    - An added benefit is the individual might shed vaccine microbe to other individuals, indirectly vaccinating them.
  - There is a possibility that the weakened pathogen could revert to a more pathogenic state, however.
  - One of the polio vaccines is a prime example of this and will be discussed shortly.

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Immunization and vaccines:

- Vaccine design: Inactivated vaccines
  - Consist of whole virus/cells that have been inactivated by heat or chemicals
    - Benefits include that the microbe can't revert, can't replicate, and can't spread.
    - Drawbacks include lower/shorter stimulation of immune responses, a need for multiple injections, and greater risk of negative side effects.
  - An example of an older vaccine (no longer in use) was killed whole-cell pertussis vaccine.
    - Crude prep of microbial components
    - Induced convulsions in 0.1% of infants (with a smaller percentage suffering brain damage)
    - Newer vaccine is a safer acellular subunit prep.

Immunization and vaccines:

- Vaccine design: Subunit vaccines
  - Consist of 1+ isolated protective antigens (no whole cells or viruses)
    - Defined composition is safer
    - May require several injections to produce strong immunity
    - The current DTaP (or Tdap) vaccine (diphtheria, tetanus, and pertussis) is an example of this type.
  - Conjugate vaccines are a modified form of this method.
    - They link a polysaccharide antigen to an immunogenic protein.
      - The idea is that the polysaccharide is a poor antigen on its own.
      - When you link it to a strong stimulating protein, you can get better responses overall.
      - The Hib vaccine, protecting against *H. influenzae* type b bacteria that cause meningitis, is an example.

Immunization and vaccines:

- Vaccine design: DNA vaccines
  - Consist of a cloned gene(s) in a DNA vector
    - Delivered to cells by injection, engineered virus, or electroporation
  - If the gene is picked up and expressed, it stimulates a protective immune response.
    - Similar to recombinant subunit vaccines
    - Benefit is longer exposure (stronger response).
    - Vaccines don't contain live microbes, avoiding the dangers associated with attenuated vaccines.
  - Largely still experimental
    - None available yet for use in human beings

Immunization and vaccines:

- Vaccine efficiency
  - No vaccine is 100% effective, but efficacy has been studied extensively.
    - Childhood vaccine effectiveness rates are usually 85–90%.
    - Estimating infected individuals in a population becomes a numbers game.
      - 1,000 individuals, all vaccinated, exposed to measles virus—how many will fall ill?
      - Now ramp this number up to a rough estimation of the population of the U.S. (375 million)—How many people might fall ill in a measles epidemic, even if we had 100% of the individuals vaccinated?
    - Opponents of vaccination use these numbers to argue that vaccines do not work—which is a misrepresentation.
    - Imagine if no one was vaccinated, instead...

Microbiology for Nursing students

Chapter Seven A: Viral Pathogenesis
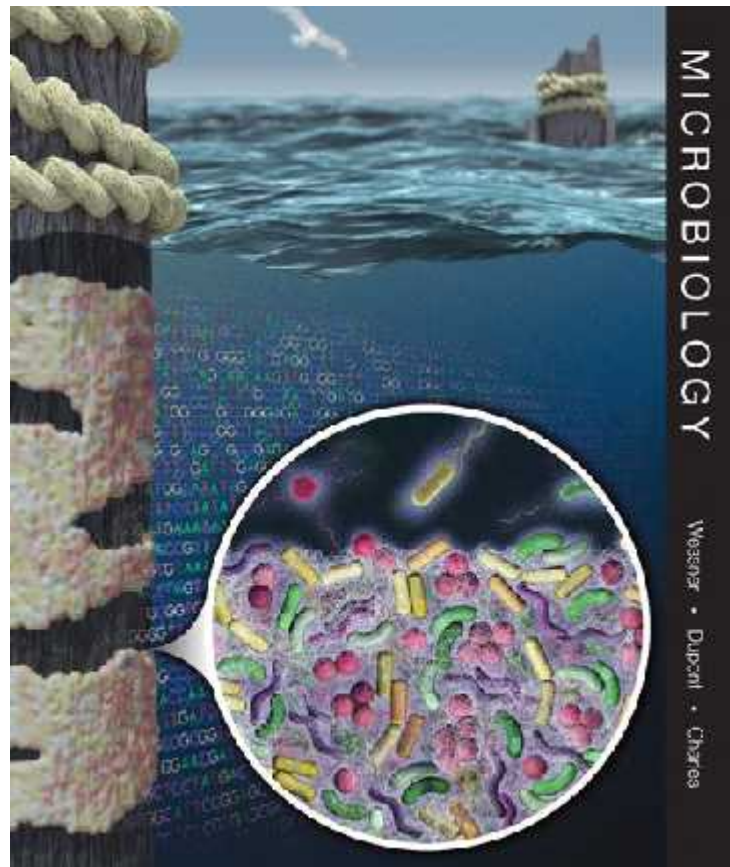

Dr. Sulaiman Alnaimat 2014

A basic overview of viruses:

- *What is a virus?*
  - History of virology
  - Viral diseases have plagued humans since before we even knew what they were (smallpox in Egypt).
  - It began as a science in late 1800s, when infectious tobacco mosaic virus was isolated in a filtered, bacteria-free fluid by Ivanovski, then Beijerinck.

© Patrick Landmann/Getty Images, Inc.

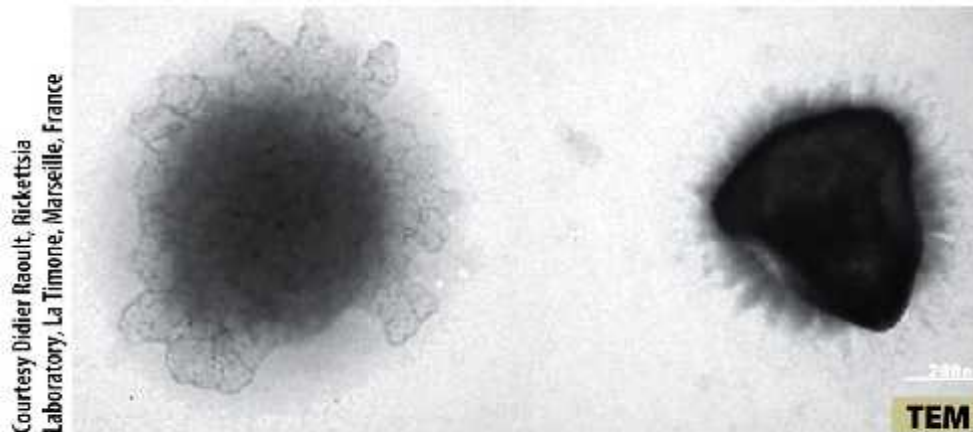# A basic overview of viruses:

- Structure of viruses: Viruses are
  - Intracellular obligate parasites
  - Typically between 10 and100 nm
  - Genomes typically between a few thousand to 200,000 nucleotides in length

3
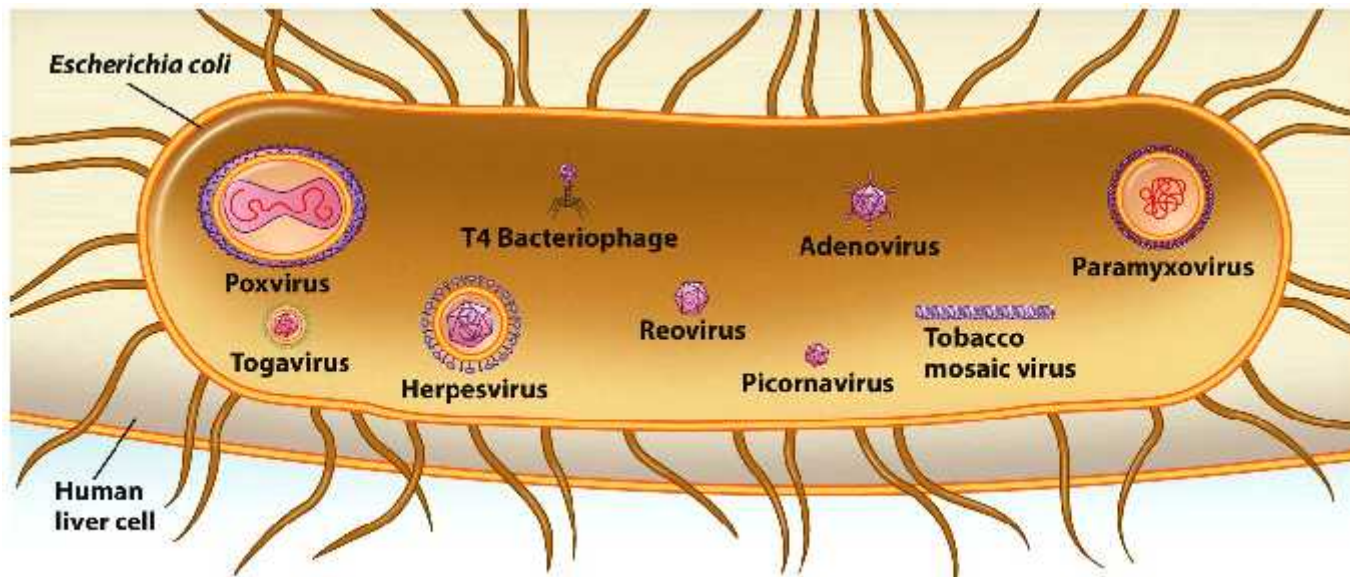
A basic overview of viruses:

- There ARE exceptions to the small size of viruses.
  - CroV virus of marine single-celled organisms has a 730k bp genome!
  - *Megavirus chilensis* (a virus of amoebas) has a genome over 1.2 megabase pairs, encodes 1,200 proteins!
  - Mimivirus (a dsDNA virus of amoebas) can be 400 nm in diameter, with a 1.2 megabase pair genome coding for 979 proteins!

Courtesy Didier Raoult, Rickettsia Laboratory, La Timone, Marseille, France

Ureaplasma urealyticum   Mimivirus

TEM

4

A basic overview of viruses:

- ## Structure of viruses
  - Single or double-stranded DNA or RNA
  - Protein shell (capsid) around genome composed of many capsomere proteins
    - capsid and genome together = nucleocapsid
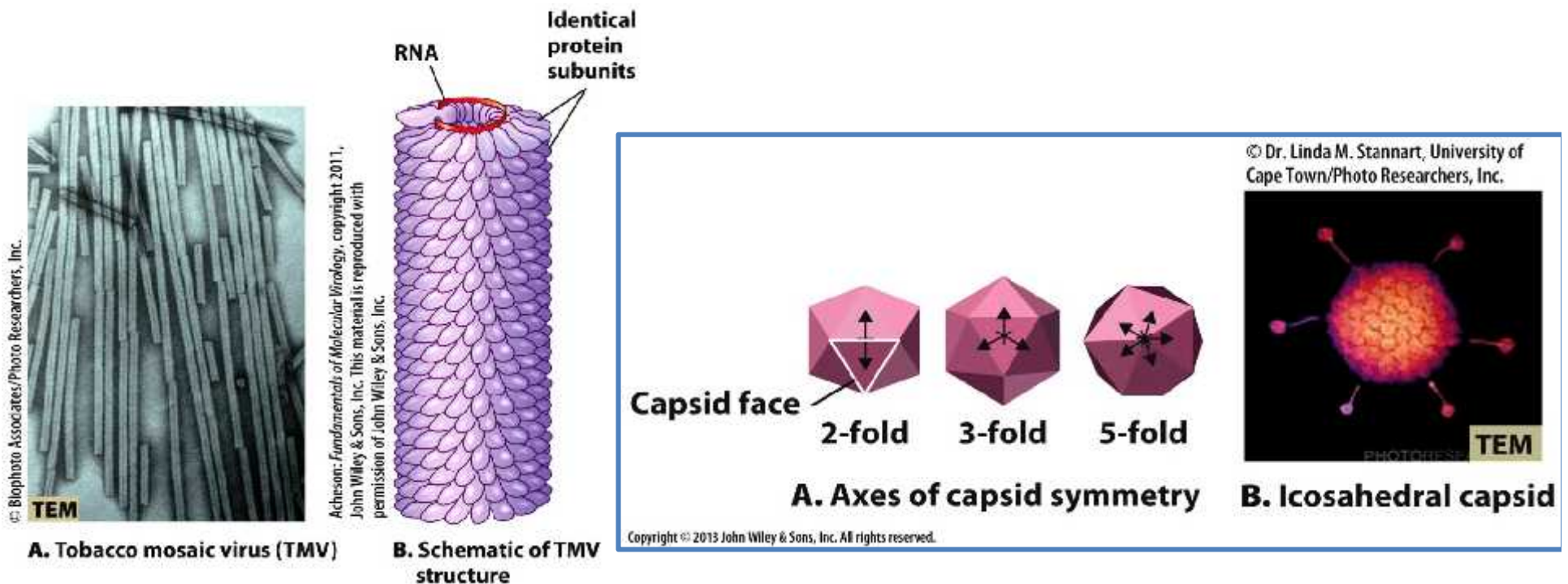 - Possible envelope (plasma membrane around capsid)

TABLE 5.1 Sizes and structural features of selected viruses

Virus	Host	Structure	Size (nm)	Genome size (bp)	Genetic material
Poliovirus (Picornavirus)	Humans	Non-enveloped, icosahedral	30 (diameter)	7,700	ssRNA
Tobacco mosaic virus (TMV)	Tobacco and related plants	Non-enveloped, helical	300 × 18	6,400	ssRNA
T4 (bacteriophage)	E. coli	Non-enveloped, complex	200 × 90	170,000	dsDNA
Smallpox virus (poxvirus)	Humans	Enveloped, complex	300 × 250	186,000	dsDNA
Mimivirus	Amoeba	Enveloped, complex	400 (diameter)	1,200,000	dsDNA

[a]ssRNA: single-stranded RNA; dsDNA: double-stranded DNA

- Structure of viruses: Symmetry
  - Capsids often exhibit either helical or icosahedral shapes.

RNA

Identical protein subunits

A. Tobacco mosaic virus (TMV)

B. Schematic of TMV structure

Capsid face

2-fold   3-fold   5-fold

A. Axes of capsid symmetry

© Dr. Linda M. Stannart, University of Cape Town/Photo Researchers, Inc.

TEM

B. Icosahedral capsid

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6

A basic overview of viruses:

- Structure of viruses: Symmetry
  - Viral capsids can sometimes take on irregular or complex shapes.

Genome ⎯⎯⎯⎯

Capsid ⎯⎯⎯⎯

Tail ⎯⎯⎯⎯

Tail fibers ⎯⎯⎯⎯

7
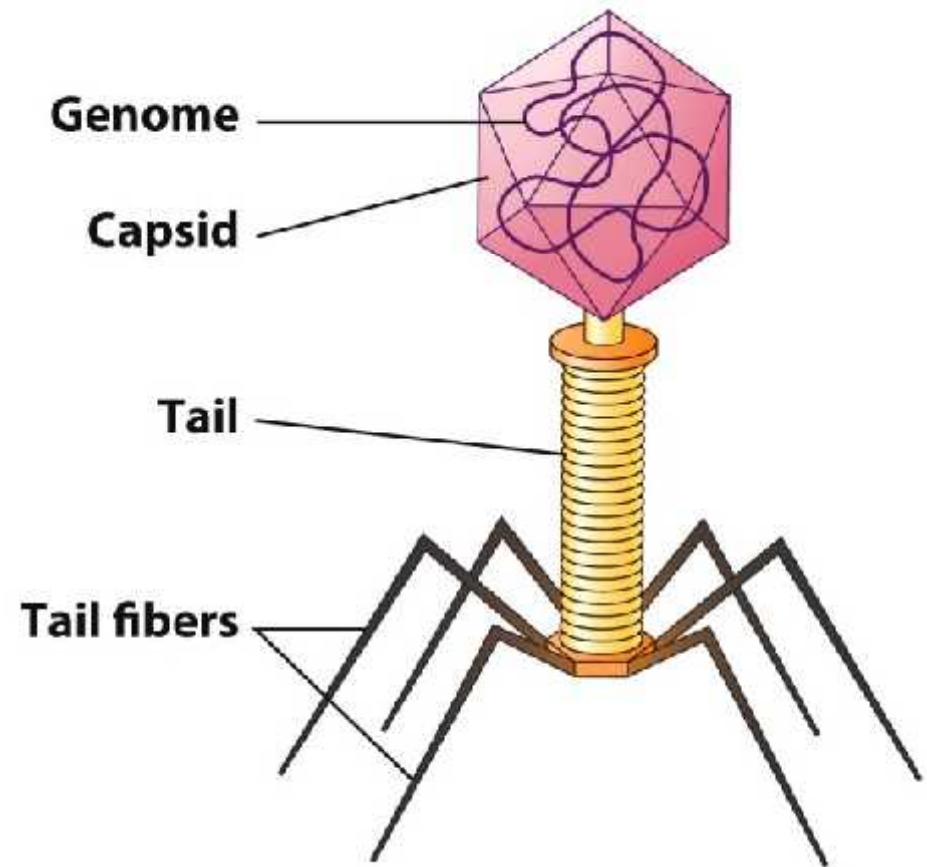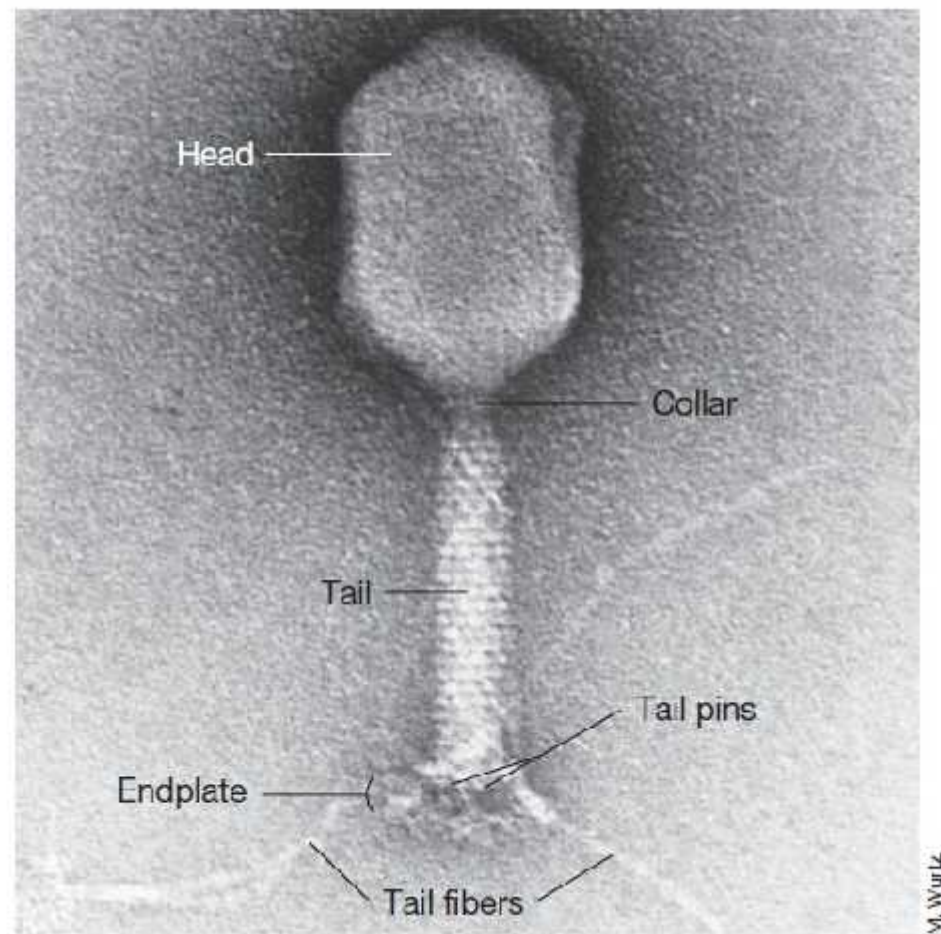
Labels on image:
Head
Collar
Tail
Tail pins
Endplate
Tail fibers
M. Wurtz
(b)

Figure 9.5 Electron micrographs of animal and bacterial viruses. (a) Influenza virus, an enveloped virus. The virions are about 80 nm in diameter, but have no defined shape (🔁 Section 21.9). (b) Bacteriophage T4 of *Escherichia coli*. The tail components function in attachment of the virion to the host and injection of the nucleic acid (Figure 9.10). The head is about 85 nm in diameter.
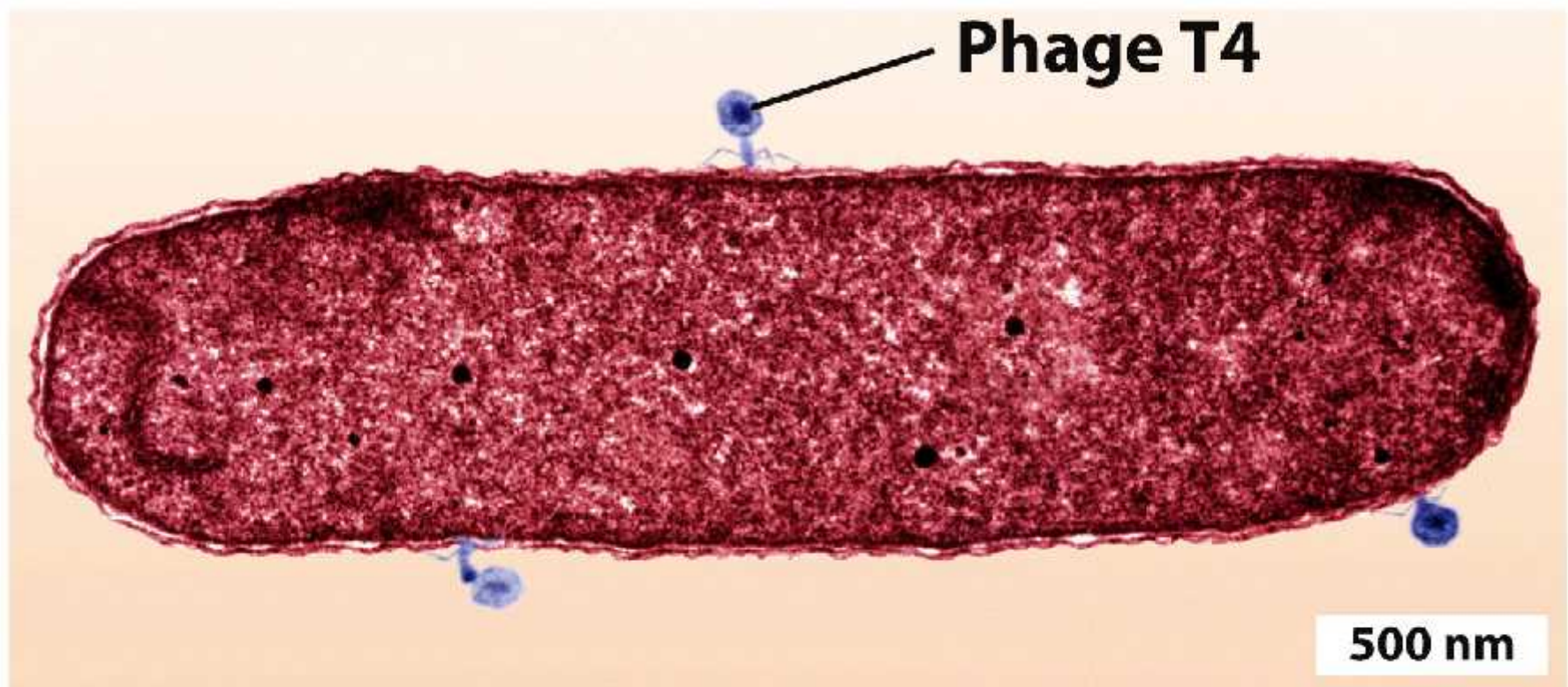
8

Phage T4

500 nm

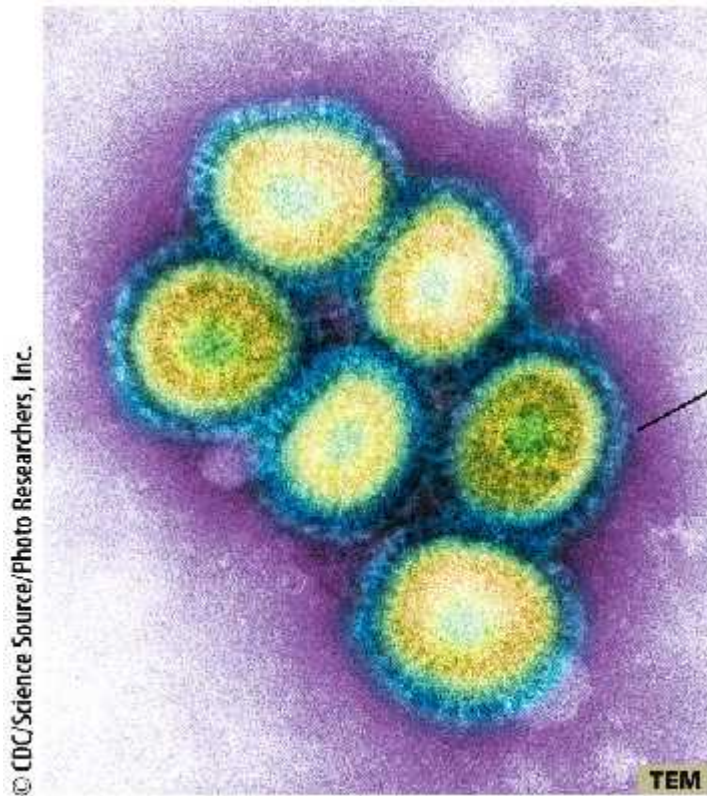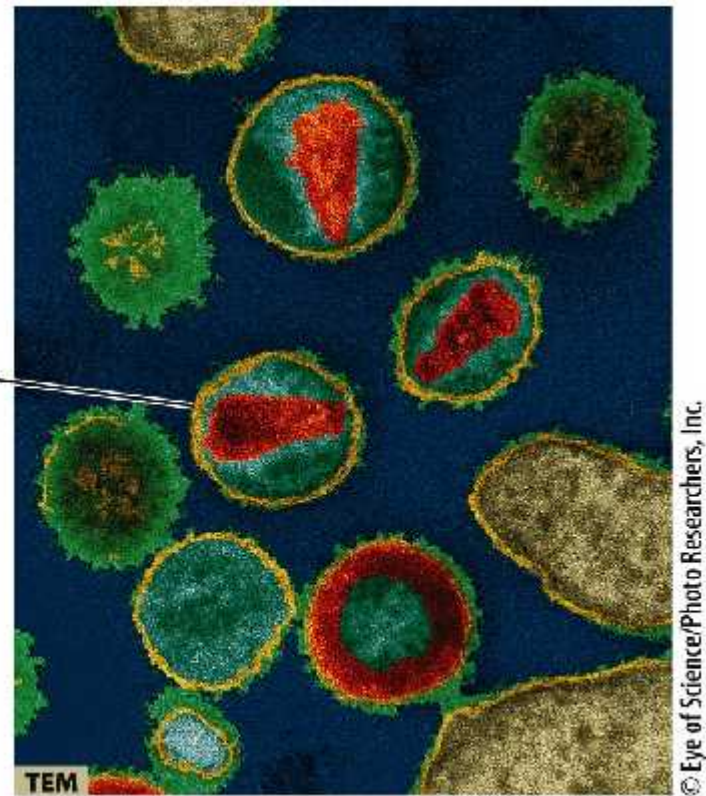Figure 6.8a Microbiology: An Evolving Science
©George Chapman/Visuals Unlimited

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- Structure of viruses: Viral Envelopes
  – If a plasma membrane surrounds the nucleocapsid, the virus is "enveloped."
  – If there is no plasma membrane, the virus is "naked."

© CDC/Science Source/Photo Researchers, Inc.

Envelope

TEM

A. Influenza virus

TEM

B. HIV

© Eye of Science/Photo Researchers, Inc.

Figure 19.3

RNA

Capsomere of capsid

Capsomere

DNA

Glycoprotein

Membranous envelope

RNA

Capsid

Glycoproteins

Head

DNA

Tail sheath

Tail fiber

18 ⎯ 250 nm

70–90 nm (diameter)

80–200 nm (diameter)

80 ⎯ 225 nm
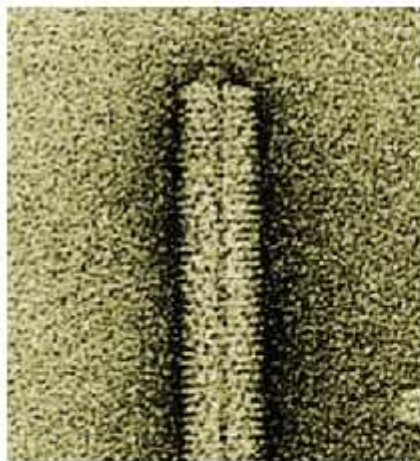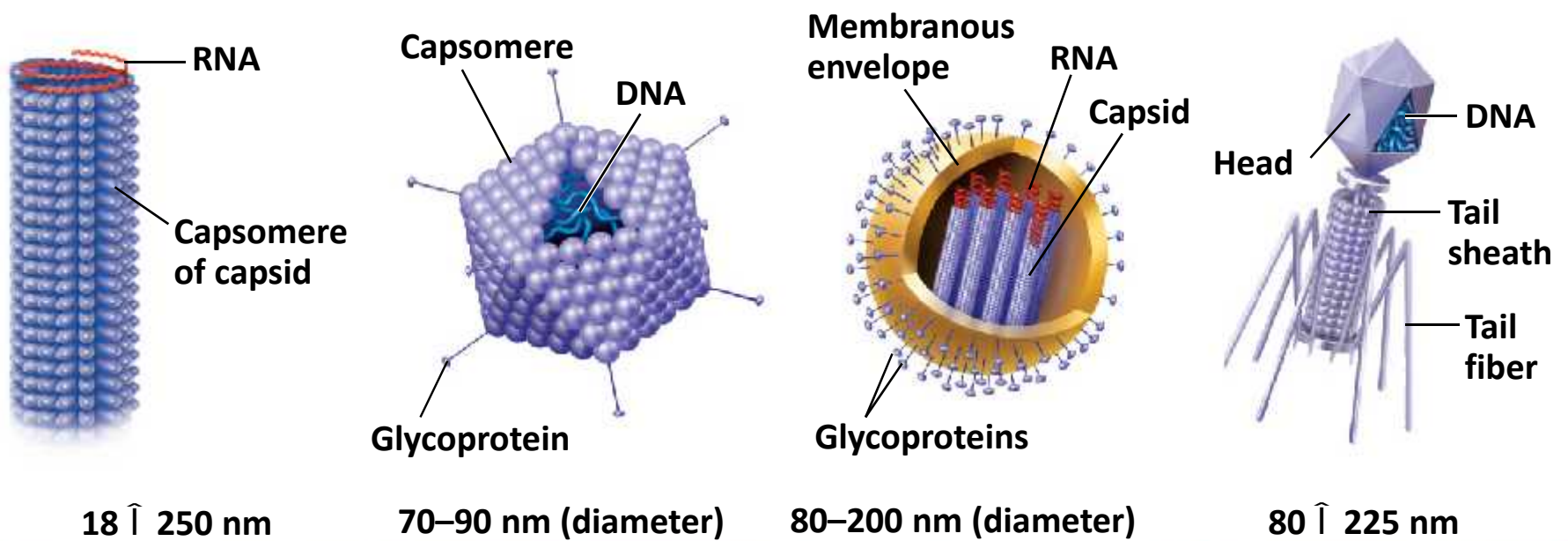
20 nm

(a) Tobacco mosaic virus
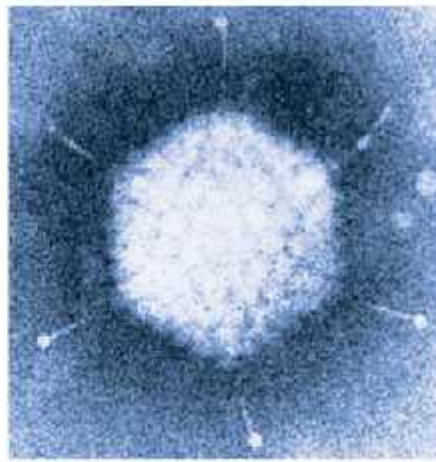
50 nm

(b) Adenoviruses
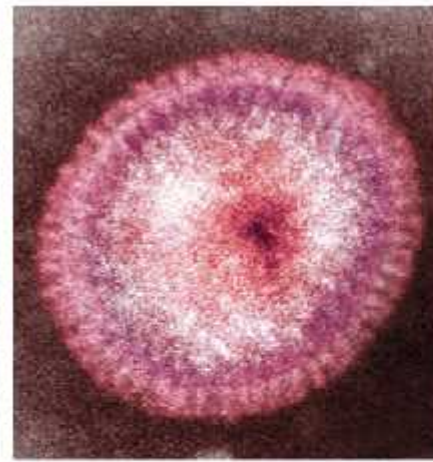
50 nm

(c) Influenza viruses
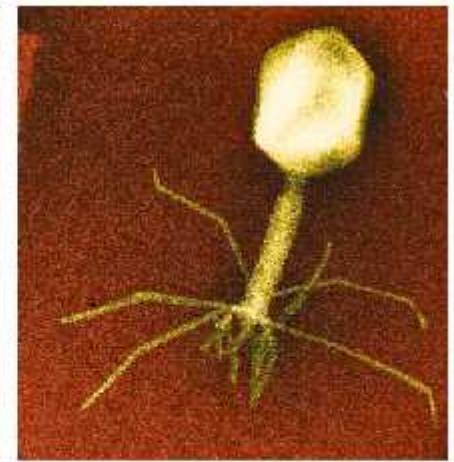
50 nm

(d) Bacteriophage T4

11

A basic overview of viruses:

- Replication cycle
  - *BRIEFLY, a virus must*
    - Stick to a host cell (adhere)
    - Get into the cell (penetrate) and release its genome (uncoat)
    - Express its genes to make proteins (synthesis)
    - Replicate its genome (synthesis)
    - Put everything together (assembly) and get the new virus particles out (exit)

Virion

① Attachment to host cell

Host cell

② Entry and uncoating of the viral genome

Viral DNA

③ Gene expression and protein production

④ Genome replication

mRNA

Viral DNA

Viral proteins

⑤ Assembly and exit from the host cell

13
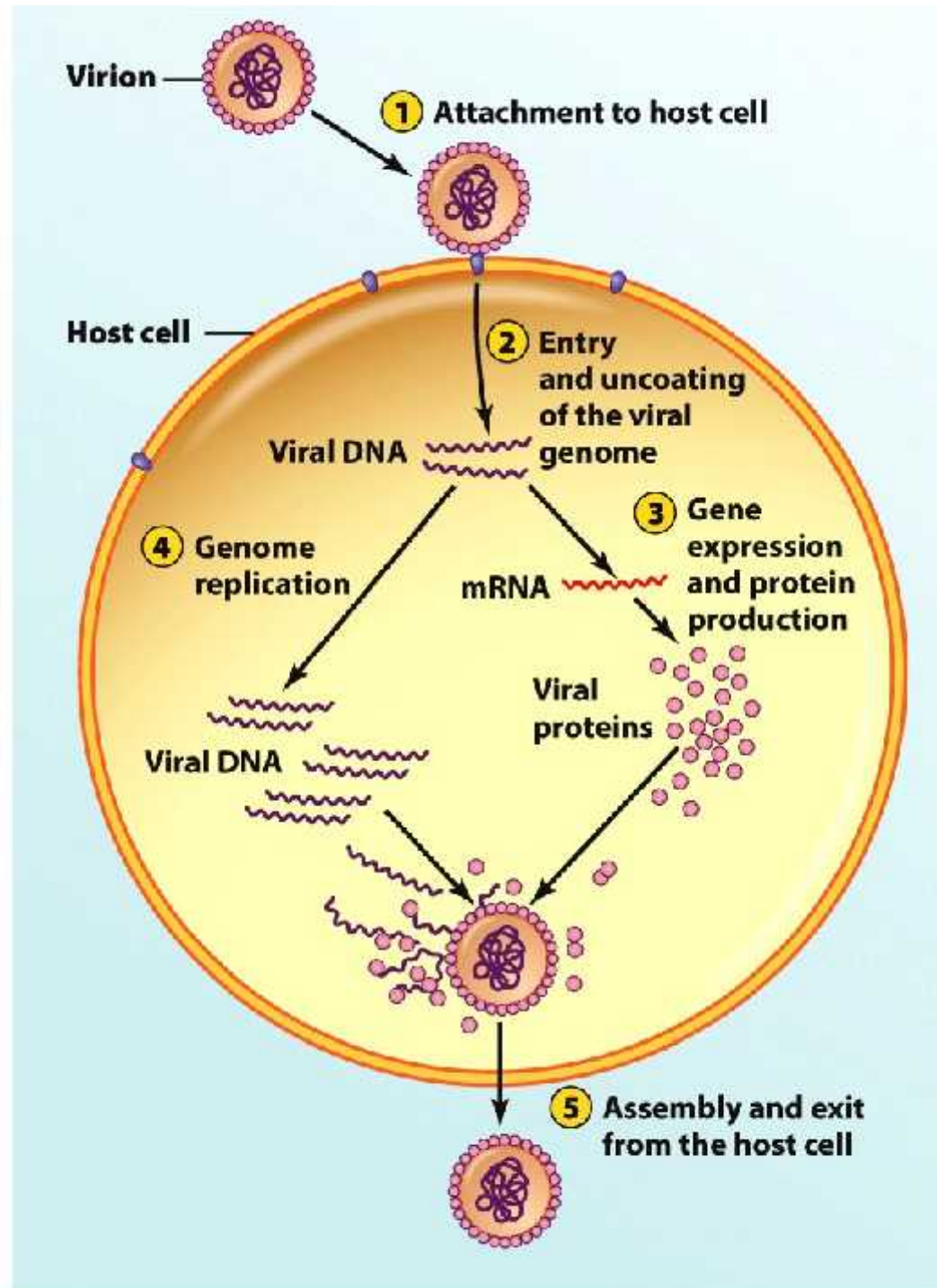
A basic overview of viruses:

- Replication cycle: Entry
  - Entry is arguably the most important part in the viral replication cycle.
  - Mechanisms for entry vary depending on the host cell.
    - Animal viruses don't have to contend with a cell wall structure.
    - Plant, fungal, and bacterial viruses do.

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1 The virus attaches to the cell receptor.

2 Endocytosis is initiated.

3 An endosome forms with the virus inside.

4 The nucleocapsid escapes to the cytoplasm and uncoats to release the genome.

Rhinovirus

Receptor

Cytoplasm
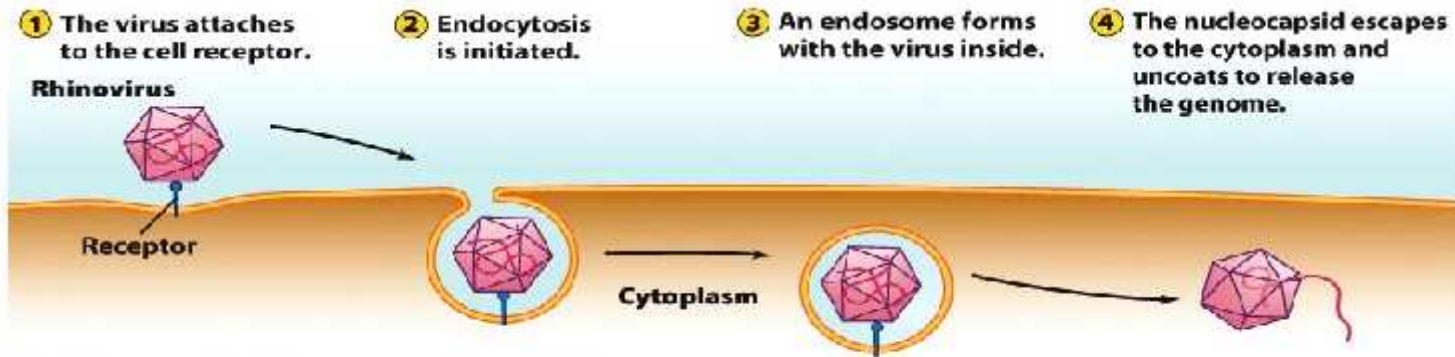
A. Endocytosis of a non-enveloped virus

1 The virus attaches to the cell receptor.

2 A conformational change in the attachment protein and bound receptor initiates membrane fusion.
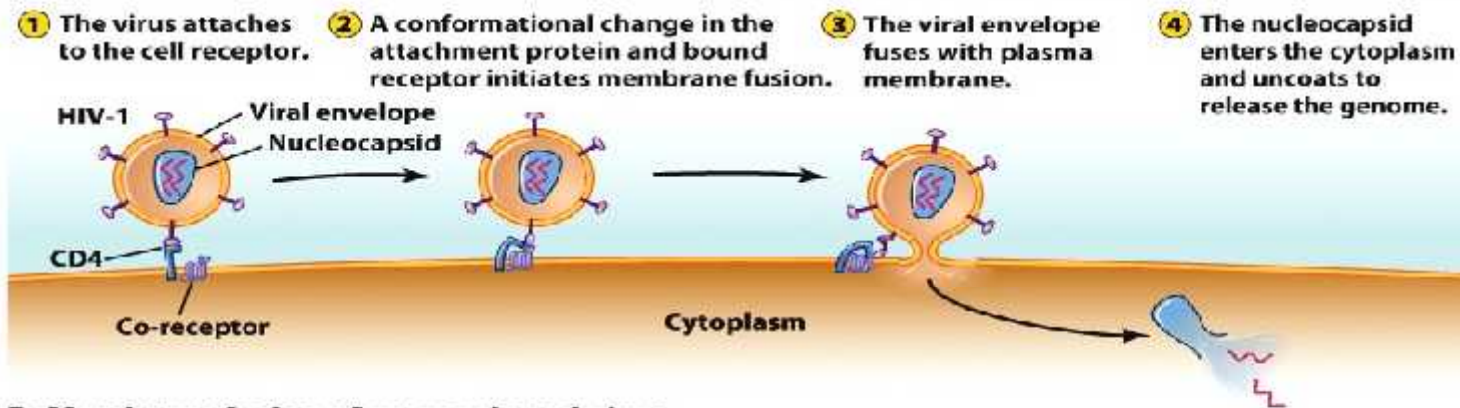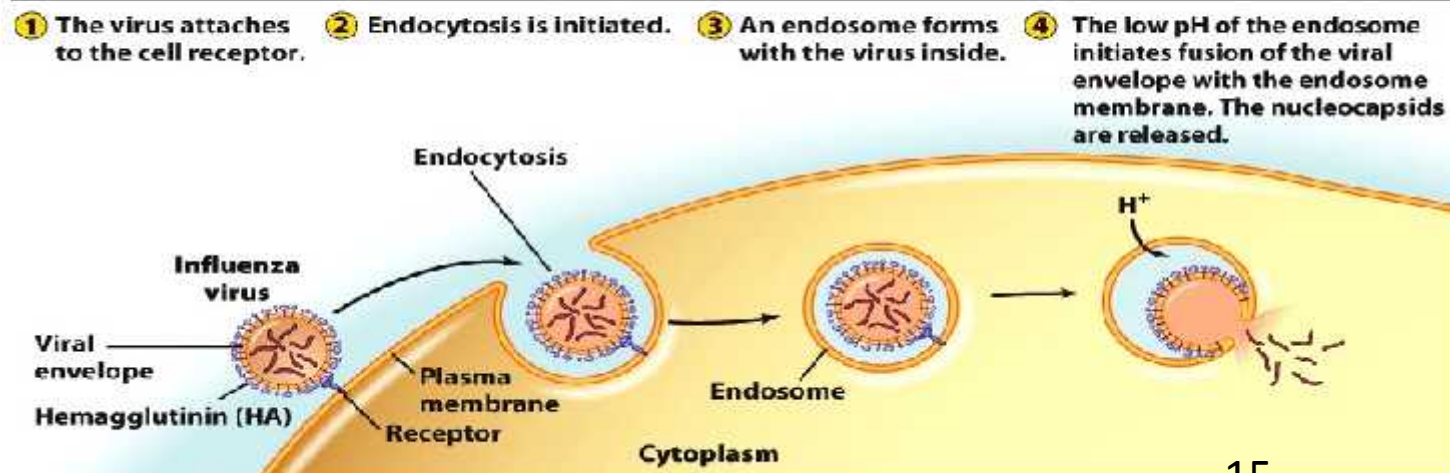
3 The viral envelope fuses with plasma membrane.

4 The nucleocapsid enters the cytoplasm and uncoats to release the genome.

HIV-1

Viral envelope

Nucleocapsid

CD4

Co-receptor

Cytoplasm

B. Membrane fusion of an enveloped virus

1 The virus attaches to the cell receptor.

2 Endocytosis is initiated.

3 An endosome forms with the virus inside.

4 The low pH of the endosome initiates fusion of the viral envelope with the endosome membrane. The nucleocapsids are released.

Endocytosis

H^+

Influenza virus

Viral envelope

Hemagglutinin (HA)

Plasma membrane

Receptor

Endosome

Cytoplasm

15

C. Endocytosis of an enveloped virus

- Replication cycle: Entry into bacteria

① Tail fibers attach to receptors.

② Conformational change in tail fibers bring base of the tail in contact with host cell surface.

③ Rearrangement of tail proteins allows inner core tube proteins to extend down into cell wall.

④ Contact with the plasma membrane initiates transfer of DNA through a pore formed in the lipid bilayer.

Plasma membrane

Core

DNA

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Figure 19.6

Phage DNA

Phage

The phage injects its DNA.

Bacterial chromosome

Phage DNA circularizes.

Daughter cell with prophage

Cell divisions produce a population of bacteria infected with the prophage.

Occasionally, a prophage exits the bacterial chromosome, initiating a lytic cycle.

Lytic cycle

Lysogenic cycle

Certain factors determine whether

The cell lyses, releasing phages.

The bacterium reproduces, copying the prophage and transmitting it to daughter cells.

lytic cycle is induced

or

lysogenic cycle is entered

Prophage

New phage DNA and proteins are synthesized and assembled into phages.

Phage DNA integrates into the bacterial chromosome, becoming a prophage.
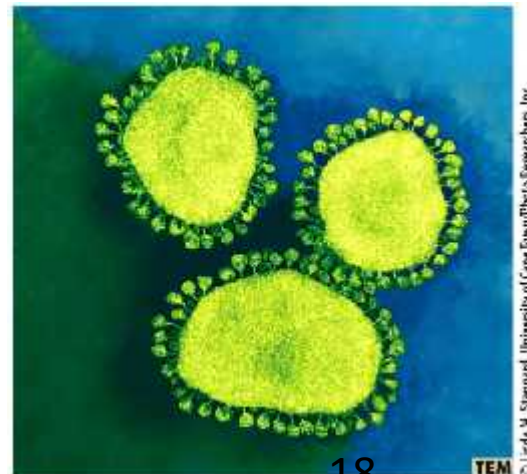
17

Diversity of viruses:

- Virus names:
  - Historically, quite varied!
    - Simple letter/number combinations (T4 phage)
    - Organism(s) they infect (tobacco mosaic virus)
    - Location of discovery (Ebola River, Zaire)
    - Appearance (coronavirus, "crown")
    - Disease caused (hepatitis viruses)

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Virus-like particles:

- *Are viruses the simplest pathogens?*
  - Viroids:
    - Consist only of naked RNA
    - are extremely small (less than 400 nucleotides)
    - Have a high degree of internal complementarity
    - Are resistant to ribonucleases
    - So far, only observed to cause disease in plants
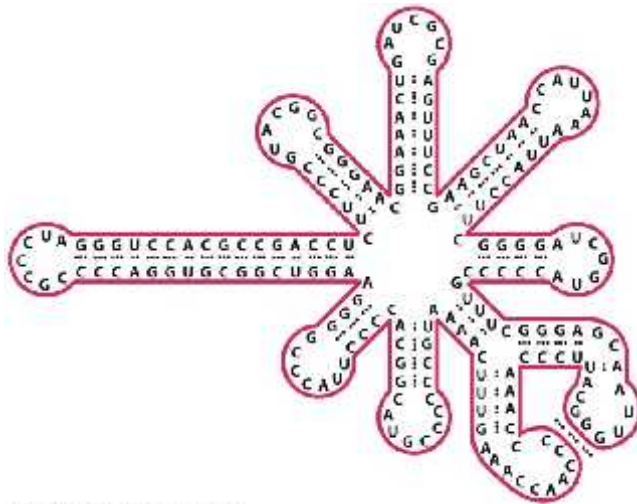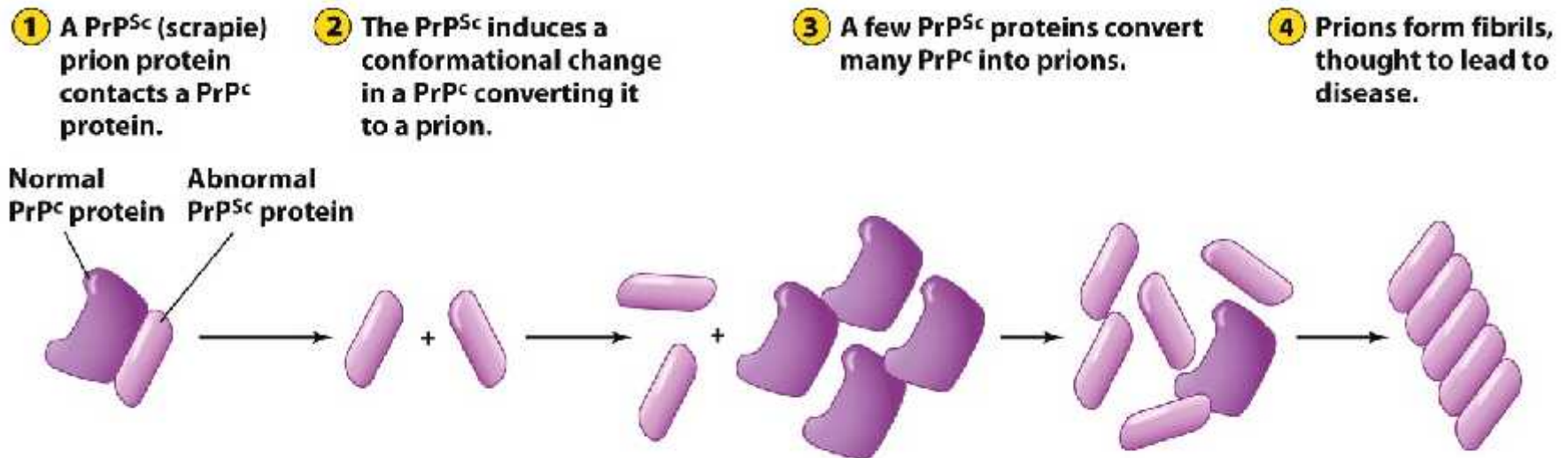

19

Virus-like particles:

- Prions (<u>pr</u>oteinaceous <u>i</u>nfectious particles):
  - No nucleic acid, no genes... just protein
  - Very different "infectious" agent
  - Responsible for transmissible spongiform encephalopathies (TSEs), such as mad cow disease
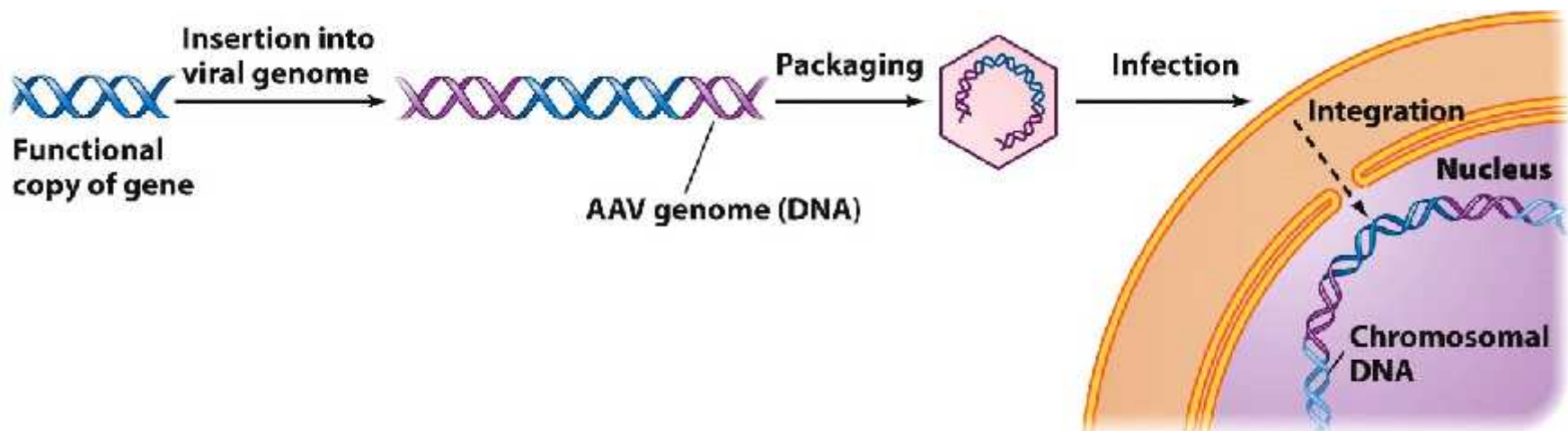

20

Virus-like particles:

- Prions (<u>pr</u>oteinaceous <u>i</u>nfectious particles):
  - Replication method still unclear
  - Thought to revolve around conversion of protein conformations from normal to abnormal form over time

① A PrP^{Sc} (scrapie) prion protein contacts a PrP^c protein.

② The PrP^{Sc} induces a conformational change in a PrP^c converting it to a prion.

③ A few PrP^{Sc} proteins convert many PrP^c into prions.

④ Prions form fibrils, thought to lead to disease.

Normal PrP^c protein   Abnormal PrP^{Sc} protein

Acheson: *Fundamentals of Molecular Virology*, copyright 2011, John Wiley & Sons, Inc. This material is reproduced with permission of John Wiley & Sons, Inc.

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Virology today:

- *What's next for virology?*
  - Unsurprisingly, virology and medicine are closely involved.
  - Virology examines cancer-causing oncoviruses.
  - Virology examines cancer-destroying oncolytic viruses.
  - Viruses can even be exploited to deliver working copies of genes to replace damaged versions (gene therapy, experimental).

Insertion into
viral genome

Functional
copy of gene

AAV genome (DNA)

Packaging

Infection

Integration

Nucleus

Chromosomal
DNA

22

Virology today:

2010: A global view of HIV infection

33.3 million people [31.4–35.3 million] living with HIV, 2009

23

Source: UNAIDS

No data   <0.1%   0.1%–<0.5%   0.5%–<1.0%   1.0%–<5.0%   5.0%–<15.0%   15.0%–28.0%
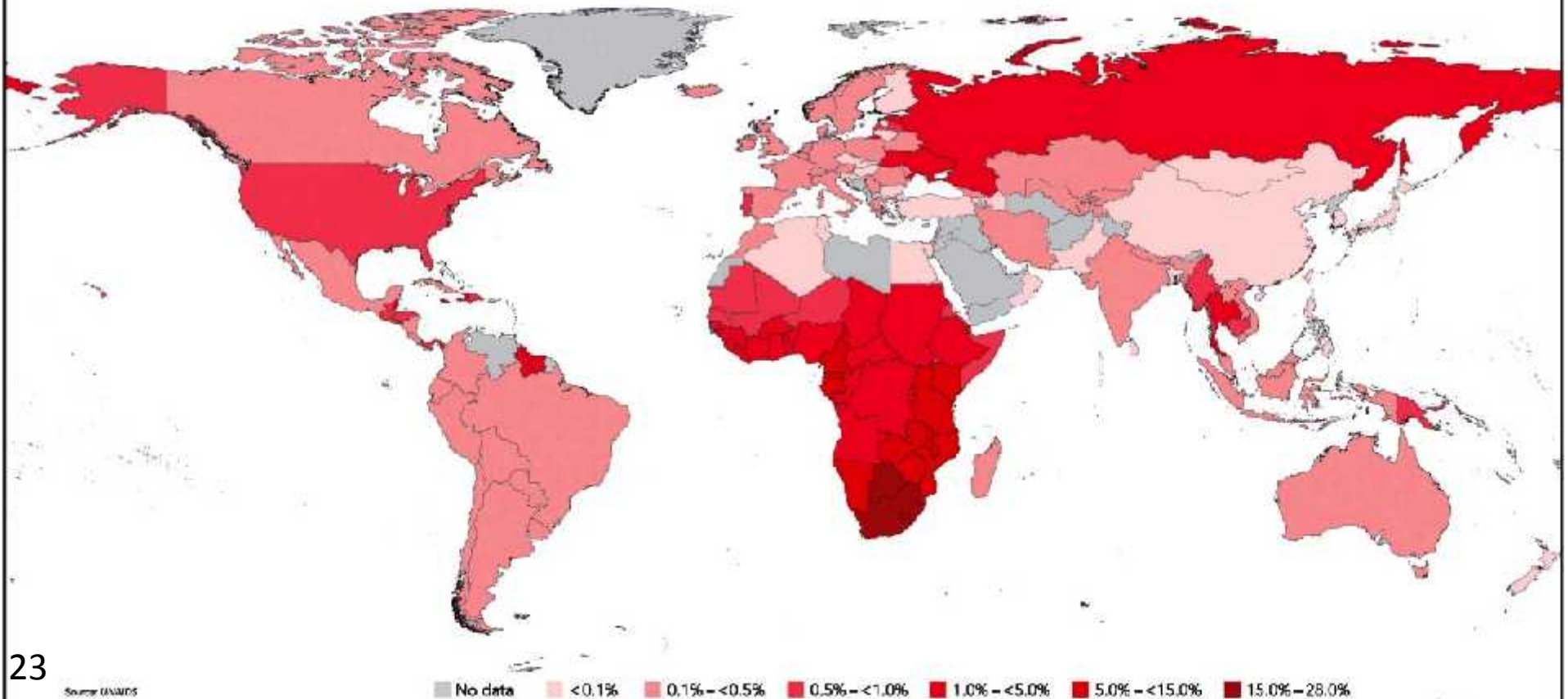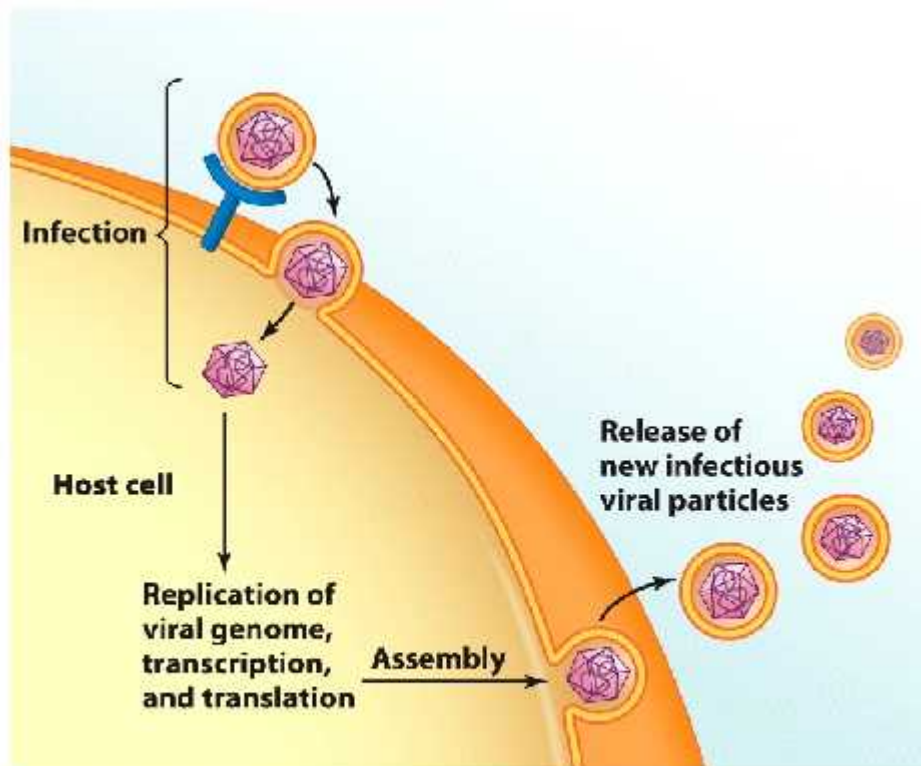
Viral Pathogenesis

- Viral pathogenesis can be highly varied and complicated.
- To reproduce, viruses must
  - Get into a permissive host
  - Acquire resources needed for replication
  - Evade host defenses
  - Spread to new hosts
- Some of these issues are similar to bacterial pathogenesis—but viruses MUST replicate intracellularly, which can complicate things.
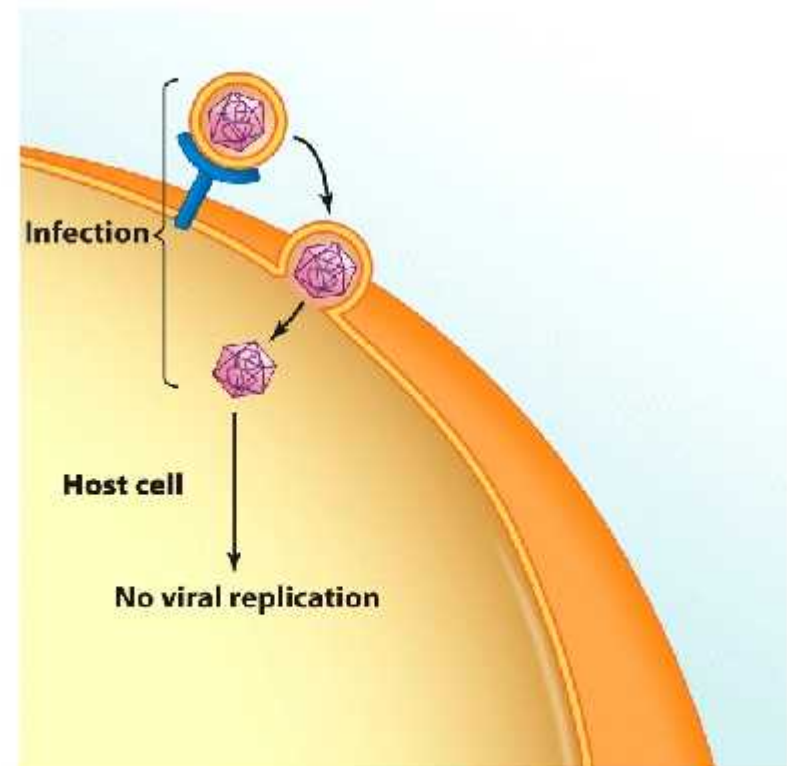
24

Recurring themes in viral pathogenesis:

- *How do viruses cause disease?*
  - With viruses, infection simply means entry of a virus into a host cell.
    - Productive infections = New infectious viral particles produced
    - Abortive infections = Few, if any, new viral particles produced
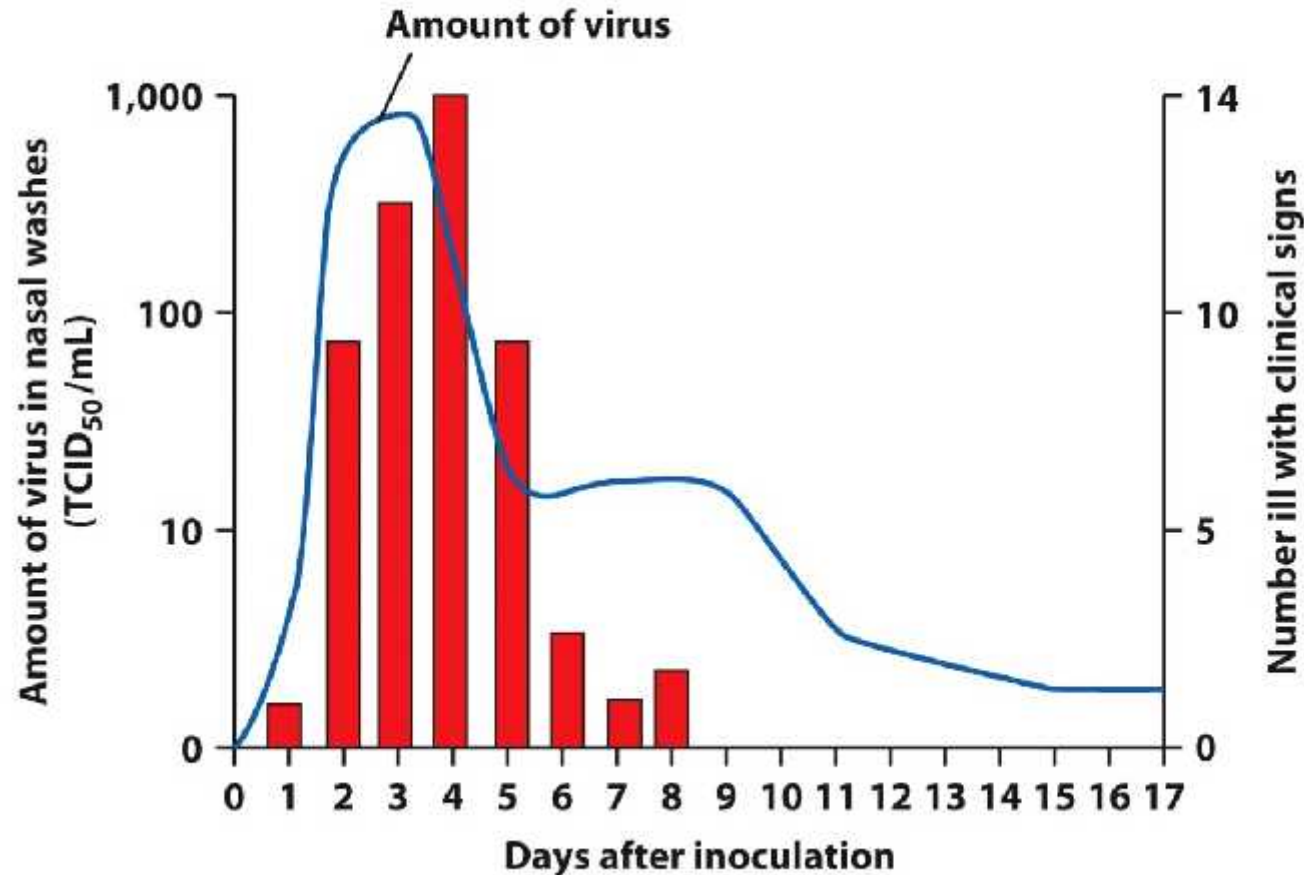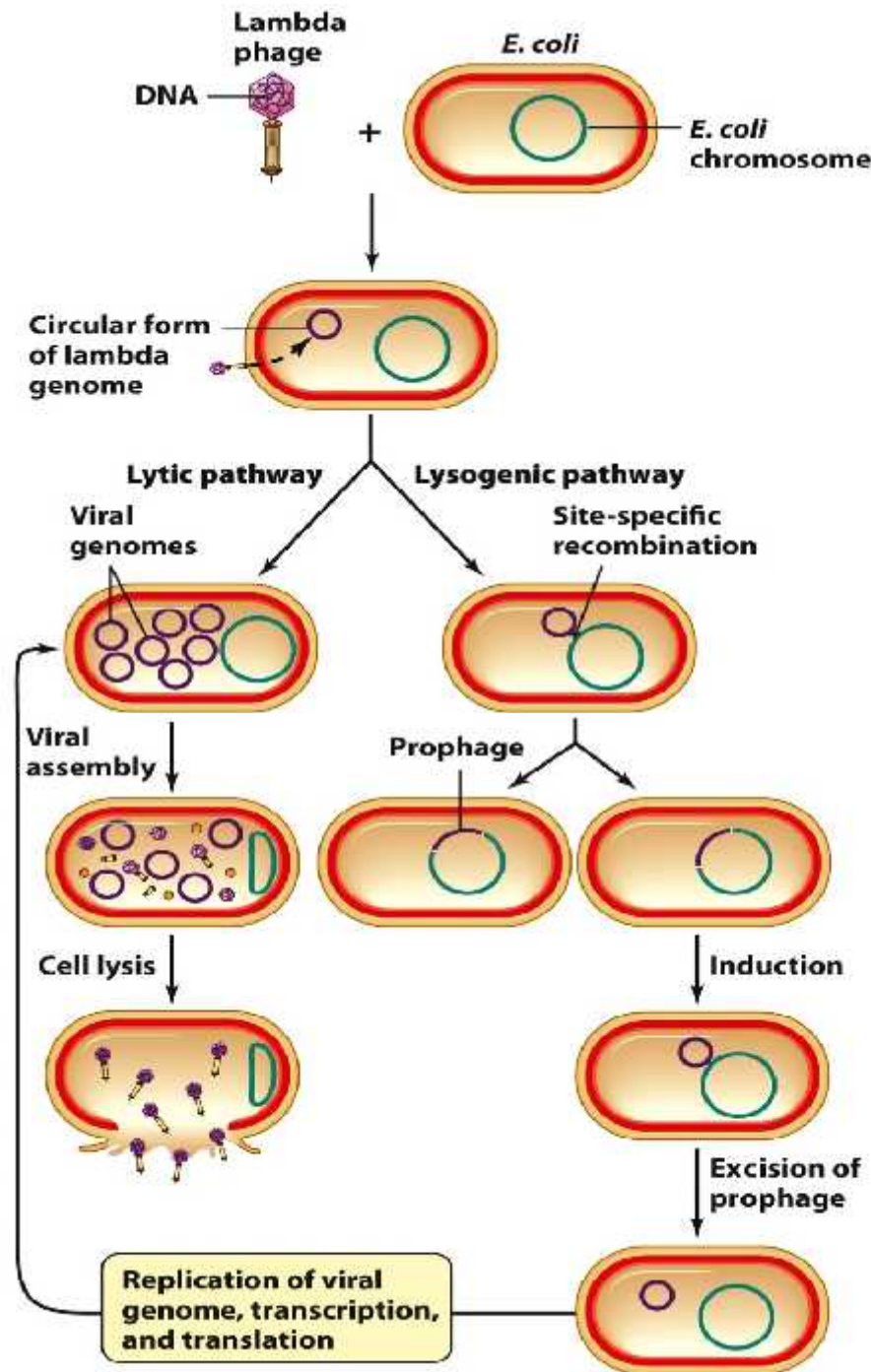

A. Productive infection: Permissive cell

B. Abortive infection: Non-permissive cell

25

Recurring themes in viral pathogenesis:

- Types of infections: Acute infections
  - Short duration, signs/symptoms observed, infection is cleared (immunity usually results)
  - Prime example is a rhinovirus infection (a common cold).

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Lambda phage
DNA
E. coli
E. coli chromosome
+

Circular form of lambda genome

Lytic pathway
Viral genomes

Lysogenic pathway
Site-specific recombination

Viral assembly

Prophage

Cell lysis

Induction

Excision of prophage

Replication of viral genome, transcription, and translation

- Types of infections: Latent infections
  - Period of acute infection is followed by latency.
    - Virus is still present, but replication is shut down.
  - Reactivation may occur, leading to recurrence of acute infection signs and symptoms.
  - Examples include lambda phage and herpesviruses.

Recurring themes in viral pathogenesis:

- Types of infections: Persistent infections
  - Aka chronic infections
  - New viral particles are continuously produced.
    - The host doesn't clear the virus, but signs and symptoms may cease.
    - This impairment may be a result of a targeted weakening of the immune system or mutational changes in the virus and/or host target cells.
  - Examples include hepatitis B and C.
    - These illnesses don't ALWAYS become persistent but CAN do so in some individuals.

- Types of transmission
  - Viruses must be able to leave one host and enter another.
  - There are several basic types of transmission.
    1. Horizontal transmission
    2. Vertical transmission
    3. Zoonotic transmission
    4. Mechanical transmission

- Types of transmission: Horizontal
  - Transfer from individual to individual within the same species
  - Requires a mode of exit and a mode of entry
  - This is what people commonly think of when they think of "catching" something from someone.

| TABLE 22.2 | Common horizontal transmission strategies | |
| --- | --- |
| **Transmission route** | **Examples** |
| Respiratory | Rhinovirus, influenza virus |
| Fecal-oral | Poliovirus, hepatitis A virus |
| Sexual | HIV, human papillomavirus |

- Types of transmission: Vertical
  - Still transmission within the same species but from mother to fetus or newborn
    - Virus may be transmitted through the placenta or during birth (rubella, hepatitis B and C, HIV).
    - Virus might also be transmitted via breast milk (HIV).

Germ cells of BALB/c mouse contain MMTV provirus.
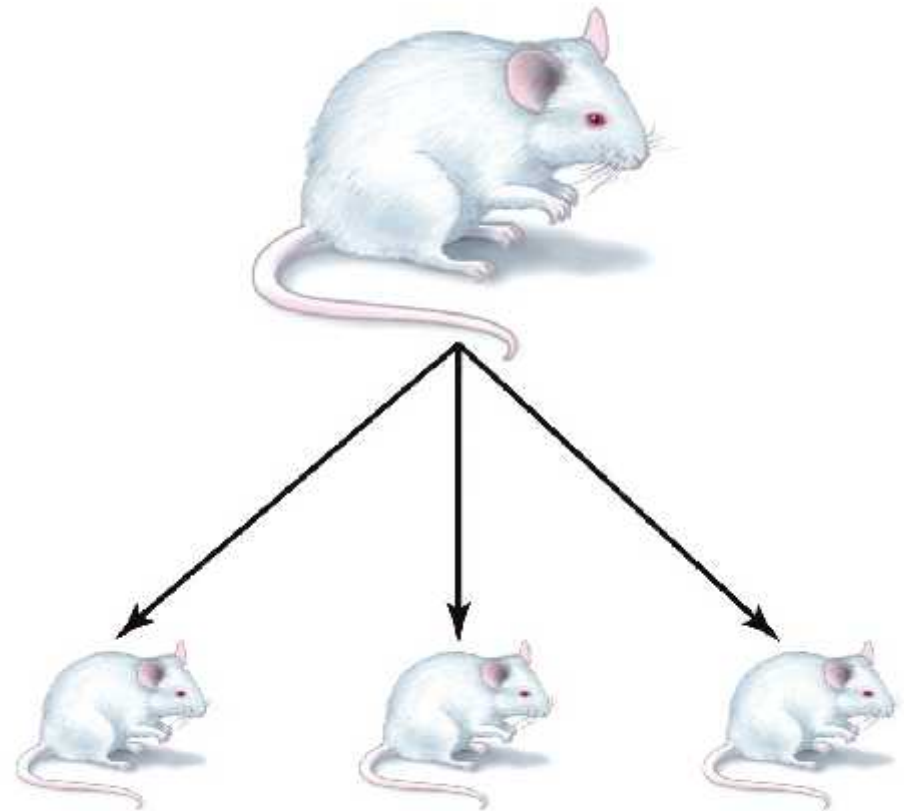
Offspring contain MMTV provirus in all cells.

- Types of transmission: Zoonotic
  - Transmission from a non-human host to humans
    - Examples include rabies, West Nile, and Ebola
    - May lead to more widespread horizontal transmission events (HIV arising from SIV)

© Merlin Tuttle/BCI/Photo Researchers, Inc.

Hammerhead bat (reservoir species)

Vector species

Direct transmission

Indirect transmission

Human

Courtesy Trevor Charles

B. Possible transmission of Ebola virus

© Scott Camazine/Photo Researchers, Inc.

TEM

A. Ebola virus

© Coleman515/iStockphoto

Rabies virus

© arlindo71/iStockphoto

West Nile virus

Courtesy David Wessner

Mosquito vector

Rabbit disease caused by myxoma virus

Rabbit host

Virus replicates within mosquito vector

Human host

Human host
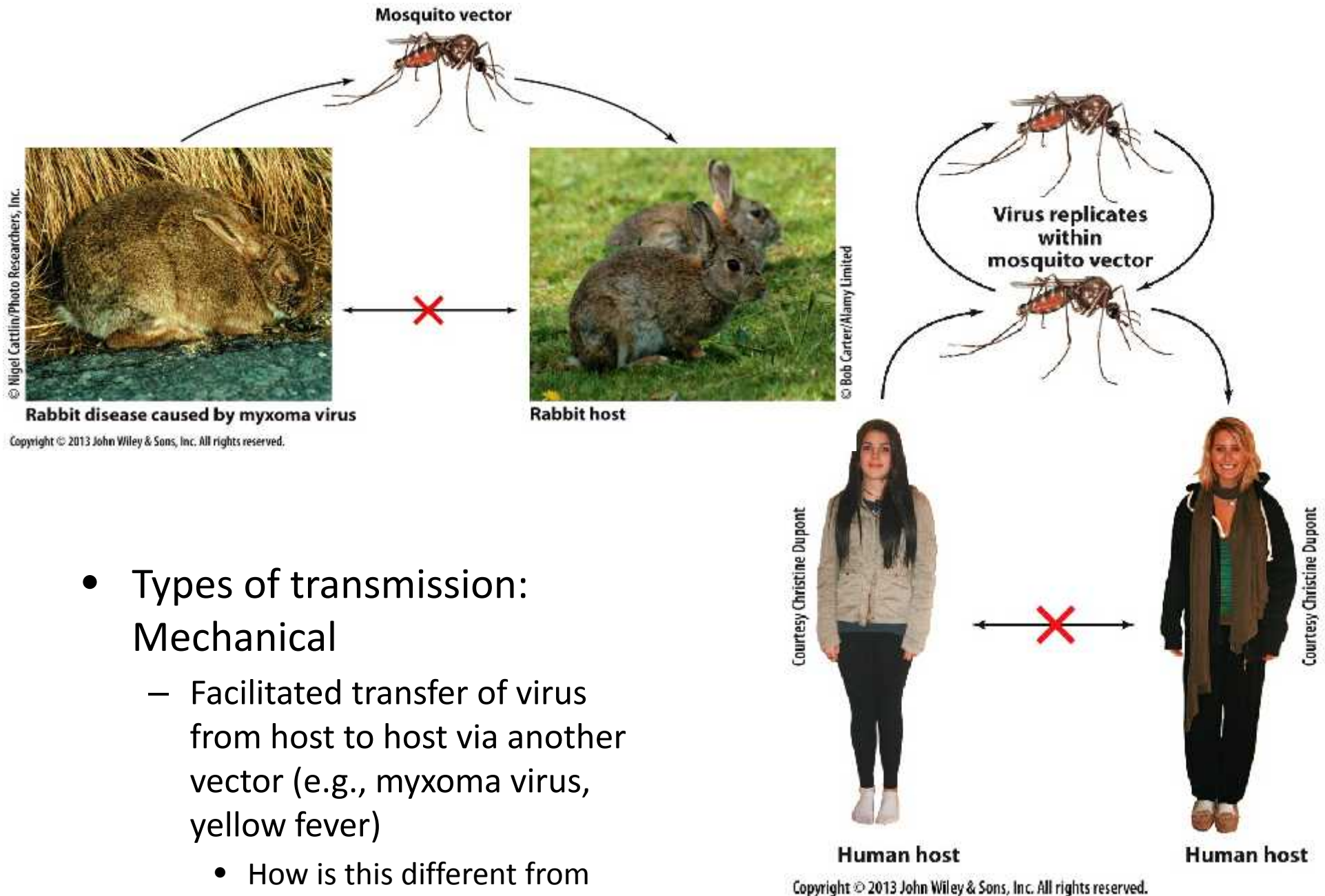
- Types of transmission: Mechanical
  – Facilitated transfer of virus from host to host via another vector (e.g., myxoma virus, yellow fever)
    • How is this different from zoonotic transmission?

Interactions with the host: Strategies and consequences

- *How do viruses interact with host cells?*
  - Viral-induced cellular destruction
    - Viruses kill cells (not always, but often).
    - There are two distinct ways in which cells will die:
      - Necrosis
      - Apoptosis
    - The outcomes of each are different and need to be defined further.

- **Viral-induced cellular destruction: Necrosis**
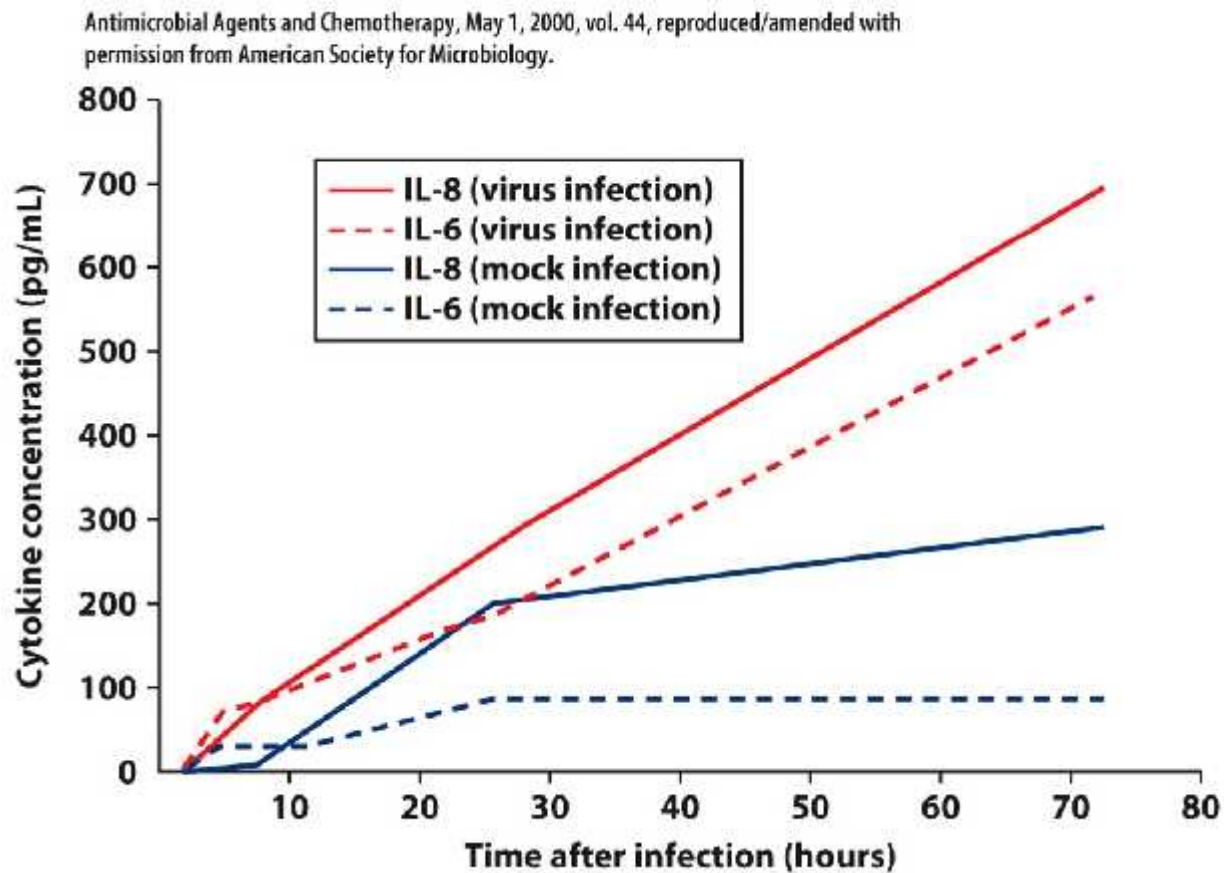 - The cell bursts.
    - Sometimes due to overfilling the cell with new viruses
    - Other times, due to viral impairment of normal cell functions
  - Two examples include poliovirus and bunyavirus cytopathology.
    - Poliovirus subverts host cell translation, causing only viral proteins to be made.
    - Bunyaviruses degrade host cell mRNA, preventing host cell proteins from being made.

- Viral-induced cellular destruction: Apoptosis
  - A more subtle death, safer for surrounding cells
  - Cell "commits suicide."
    - Internal events occur that degrade DNA into small fragments.
    - Bits of cell are released as "blebs."
    - This typically does NOT induce inflammation.
  - Induction of apoptosis is used by our immune system to safely kill virally infected cells.
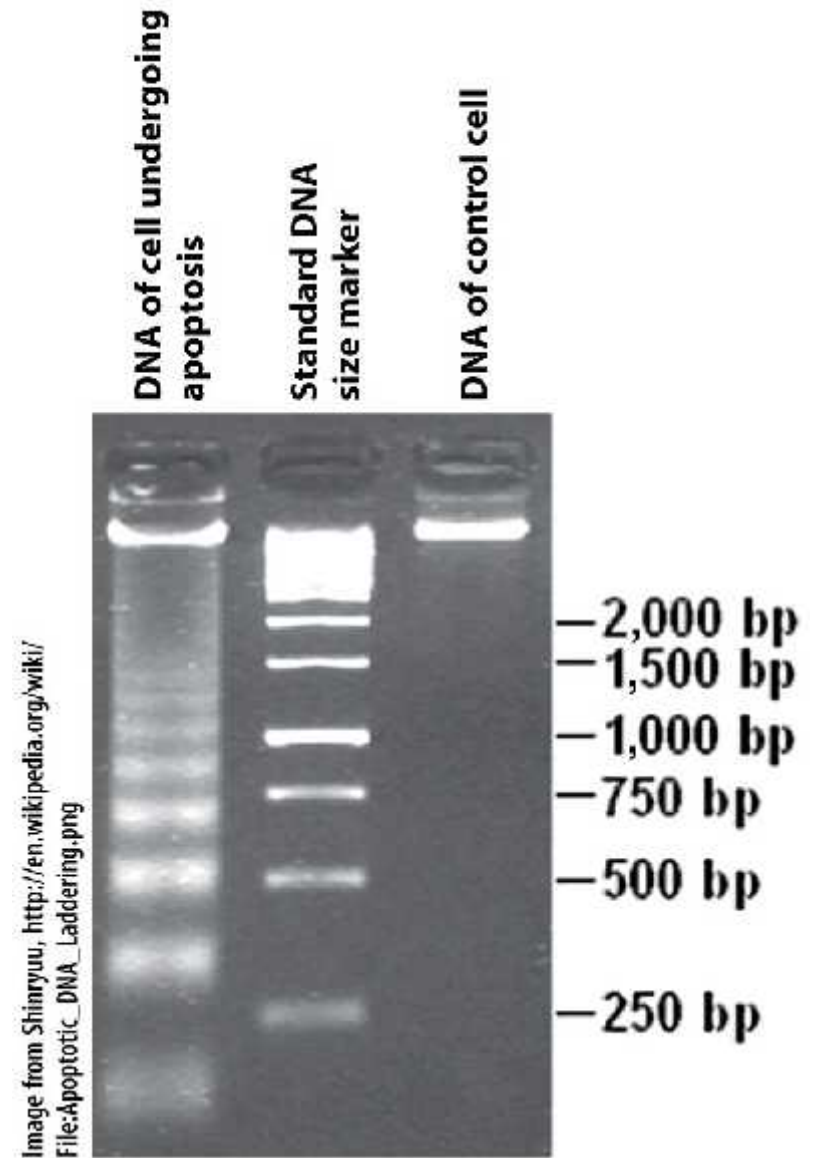
DNA of cell undergoing apoptosis

Standard DNA size marker

DNA of control cell

—2,000 bp
—1,500 bp
—1,000 bp
—750 bp
—500 bp
—250 bp

Image from Shinryuu, http://en.wikipedia.org/wiki/ File:Apoptotic_DNA_Laddering.png

- Immune responses and disease
  - Signs/symptoms of viral infection may be a result of our own immune responses.
    - Inflammatory cytokines IL-6/IL-8 in the common cold

Antimicrobial Agents and Chemotherapy, May 1, 2000, vol. 44, reproduced/amended with permission from American Society for Microbiology.

Legend:
- IL-8 (virus infection)
- IL-6 (virus infection)
- IL-8 (mock infection)
- IL-6 (mock infection)

Y-axis: Cytokine concentration (pg/mL), 0 to 800
X-axis: Time after infection (hours), 10 to 80

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© James Cavallini/ Photo Researchers, Inc.

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Hepatitis B virus (HBV)

Acute infection

Chronic infection

Clearance

Liver disease (cytotoxic lymphocyte-mediated)
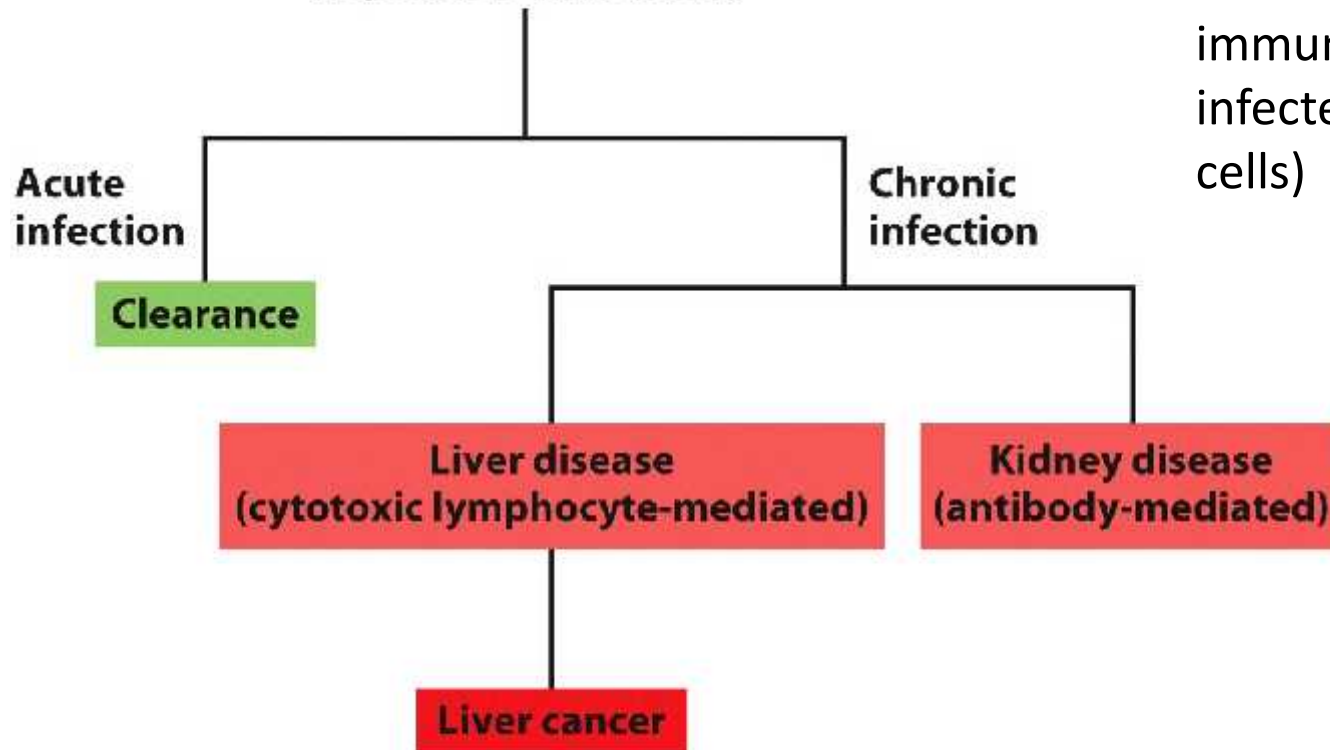
Kidney disease (antibody-mediated)

Liver cancer
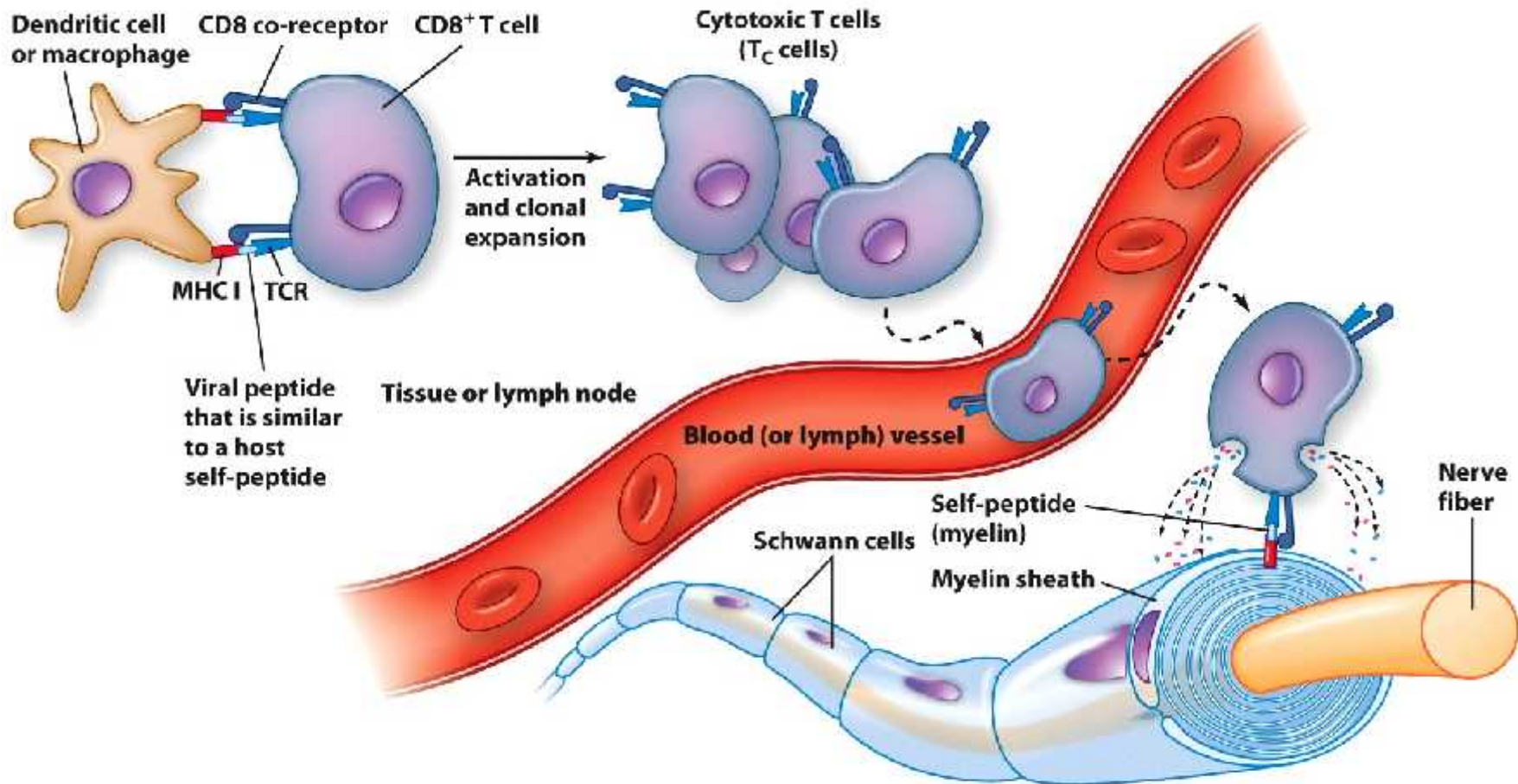
- Immune responses and disease
  - Signs/symptoms of viral infection may be a result of our own immune responses.
    - Impact of hepatitis B virus on liver function a result of immune responses killing off infected hepatocytes (liver cells)

- Immune responses and disease
  - Signs/symptoms of autoimmune diseases may be a result of our own immune responses against viruses.
    - If molecular mimicry occurs, an antiviral response accidently views self structures as foreign.

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Viral infections and cancer:

- *How do some viruses cause cancer?*
  - Transformation events produce changes in a cell that make it cancer-like.
    - Some viruses possess oncogenes, genetic material capable of inducing transforming events in host cells.
    - Tumorigenesis may result from a viral protein inducing a normally quiescent (not dividing) cell to enter the cell cycle.

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Viral infections and cancer:

- RNA tumor-causing viruses
  - Very few seem to cause cancer (mainly retroviruses).
    - Chronic hepatitis C may result in liver cancer via constant attempts to replace the damaged liver cells.
  - Retroviruses act indirectly to induce cancer.
    - Via altering proto-oncogenes (genes that code for regulation of the cell cycle)
      - *Cis*-acting retroviruses integrate their genomes, activating a cellular proto-oncogene.
      - Transducing retroviruses acquire a cellular gene and bring it with them into a newly-infected cell.

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Medical Microbiology

Chapter Seven B: Some viral infection examples
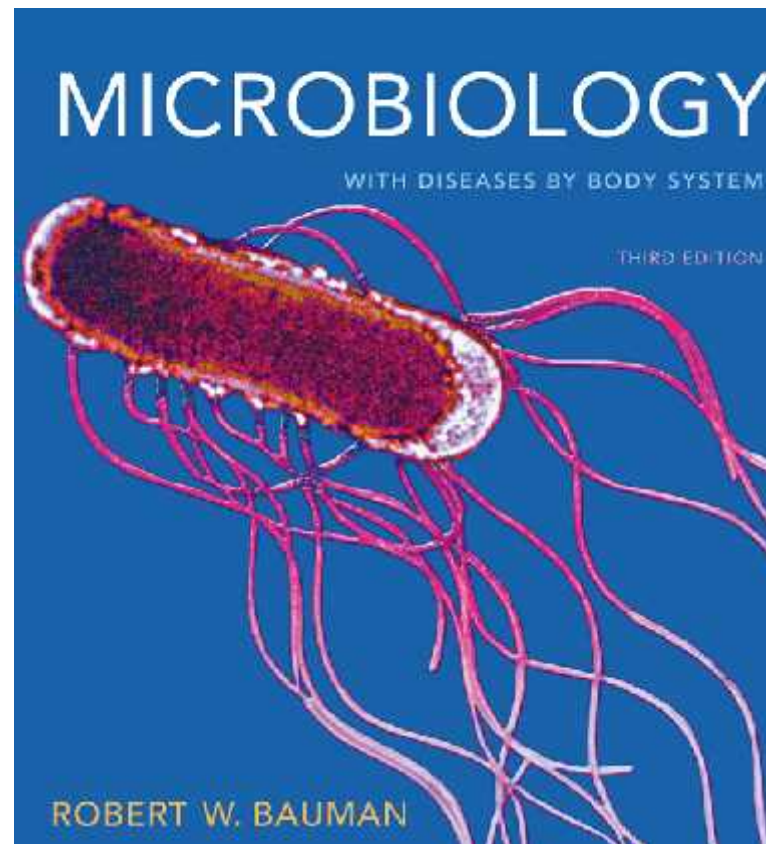
This section were taken from different references, notably **Bauman, Robert W. "Microbiology With Diseases By Body System.(3th)." (2012).**

Dr. Sulaiman Alnaimat 2014

Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - Result from a number of causes
    - Severe stress
      - Excess production of corticosteroids suppresses cell-mediated immunity
    - Malnutrition and environmental factors
      - Inhibit production of B cells and T cells
    - Acquired immunodeficiency syndrome (AIDS)
      - Opportunistic infections, low CD4 cells, presence of HIV

Figure 18.14 Diseases associated with AIDS-overview

(a)

(b)
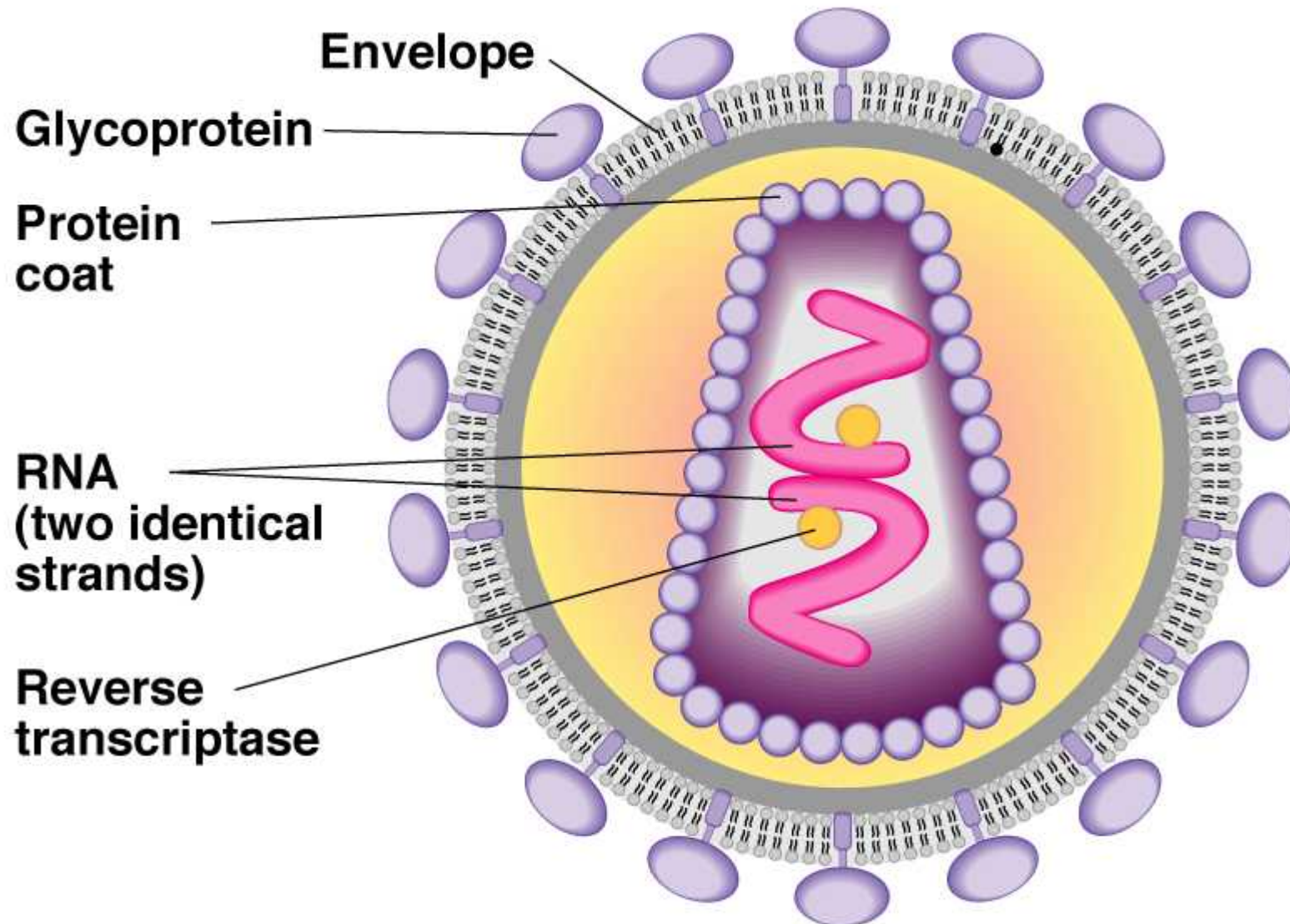
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Table 18.6 Opportunistic Infections Associated with AIDS

Opportunistic Infections Associated with AIDS

Disease	Causative Agent	Organ Primarily Affected
Coccidioidomycosis	*Coccidioides* (fungus)	Lung (22)
Cytomegalovirus disease	*Cytomegalovirus*	Brain (20), liver (23)
Diarrhea (severe and prolonged)	Various bacteria, *Cryptosporidium* (protozoan)	Intestines (23)
Herpes	*Herpesvirus*	Skin (19)
Histoplasmosis	*Histoplasma* (fungus)	Lung (22)
Kaposi's sarcoma	Human herpesvirus 8	Blood vessels (21)
Meningitis	*Cryptococcus* (yeast), *Listeria* (bacterium)	Brain and meninges (20)
Oral hairy leukoplakia	*Lymphocryptovirus* (Epstein-Barr virus)	Tongue (23)
Pneumonia	*Pneumocystis* (fungus)	Lung (22)
Shingles	*Varicellovirus*	Skin (19)
Thrush	*Candida* (yeast)	Mouth and tongue (23), vagina (24)
Toxoplasmosis	*Toxoplasma* (protozoan)	Brain (20)
Tuberculosis	*Mycobacterium*	Lung (22)

Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - AIDS pathogenesis and its virulence factors
    - Human immunodeficiency virus (HIV)
      - Retrovirus
      - Two major types
        - HIV-1 is prevalent in the United States and Europe
        - HIV-2 is prevalent in West Africa

Structure of the Human Immunodeficiency Virus HIV is a Retrovirus

Envelope

Glycoprotein

Protein coat

RNA (two identical strands)

Reverse transcriptase

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Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - AIDS pathogenesis and its virulence factors
    - Origin of HIV
      - Likely arose from mutation of the simian immunodeficiency virus (SIV)
      - Estimated to have emerged in the human population around 1930
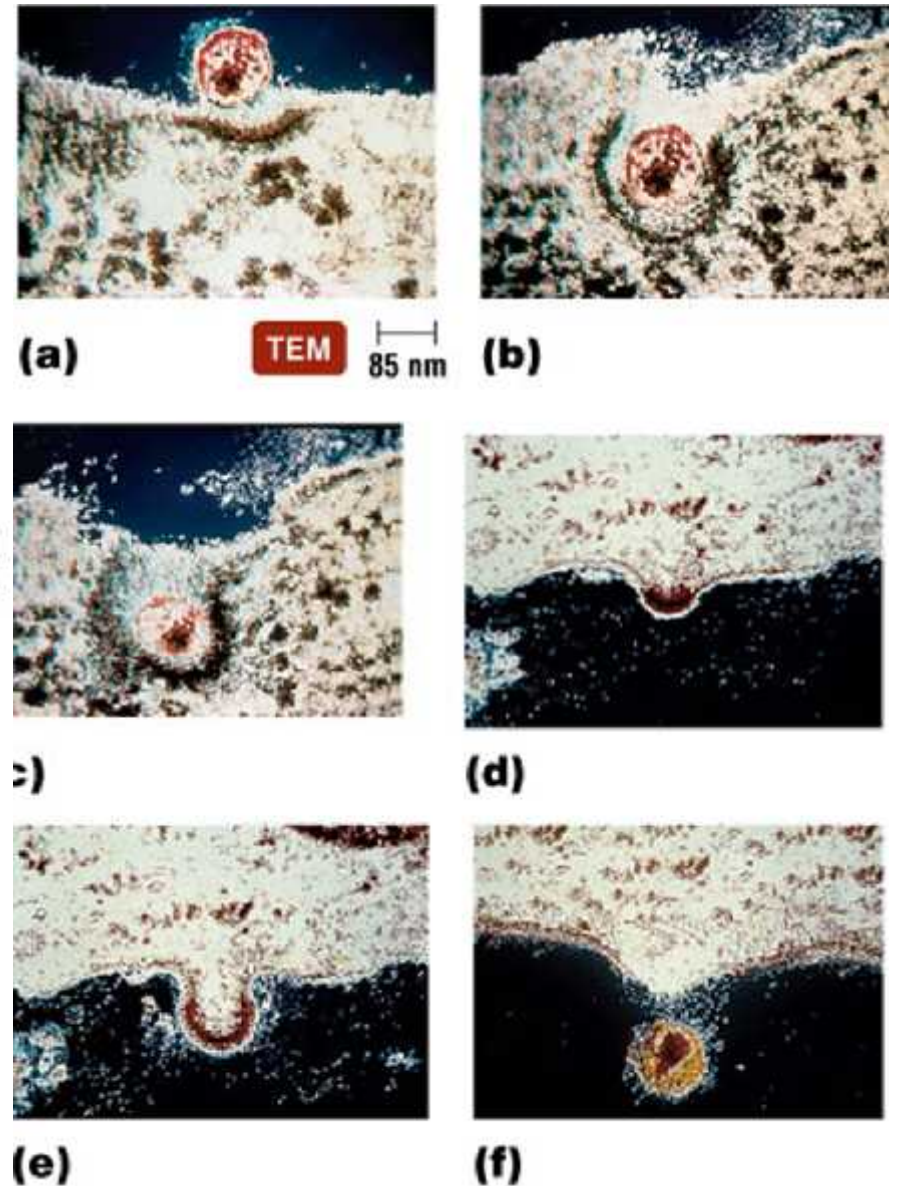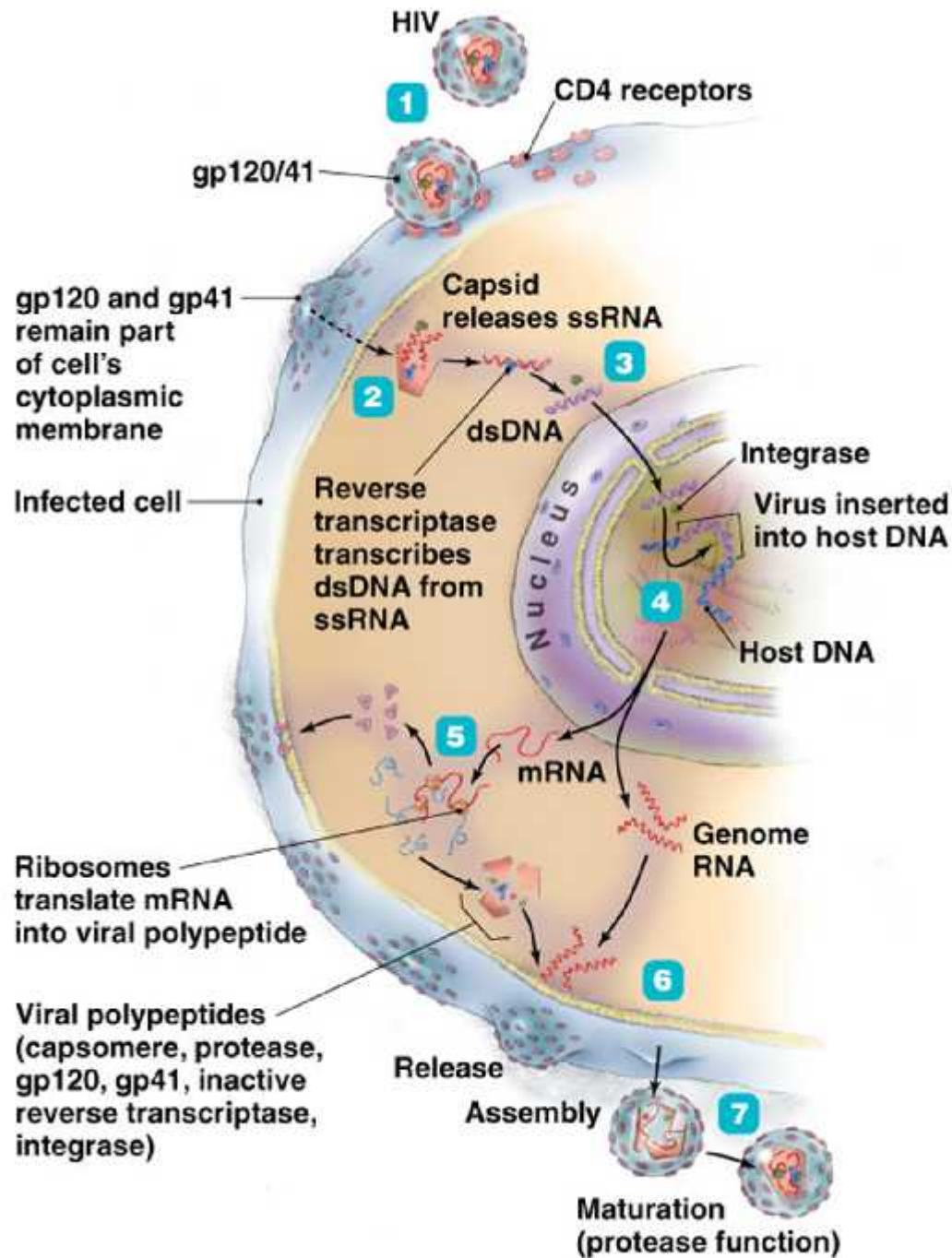
Figure 18.16 The replication cycle of HIV-overview

HIV

CD4 receptors

gp120/41

1

gp120 and gp41 remain part of cell's cytoplasmic membrane

2

Capsid releases ssRNA

3

dsDNA

Infected cell

Reverse transcriptase transcribes dsDNA from ssRNA

Nucleus

Integrase

Virus inserted into host DNA

4

Host DNA

5

mRNA

Genome RNA

Ribosomes translate mRNA into viral polypeptide

Viral polypeptides (capsomere, protease, gp120, gp41, inactive reverse transcriptase, integrase)

Release

Assembly

6

7

Maturation (protease function)

(a)

(b)

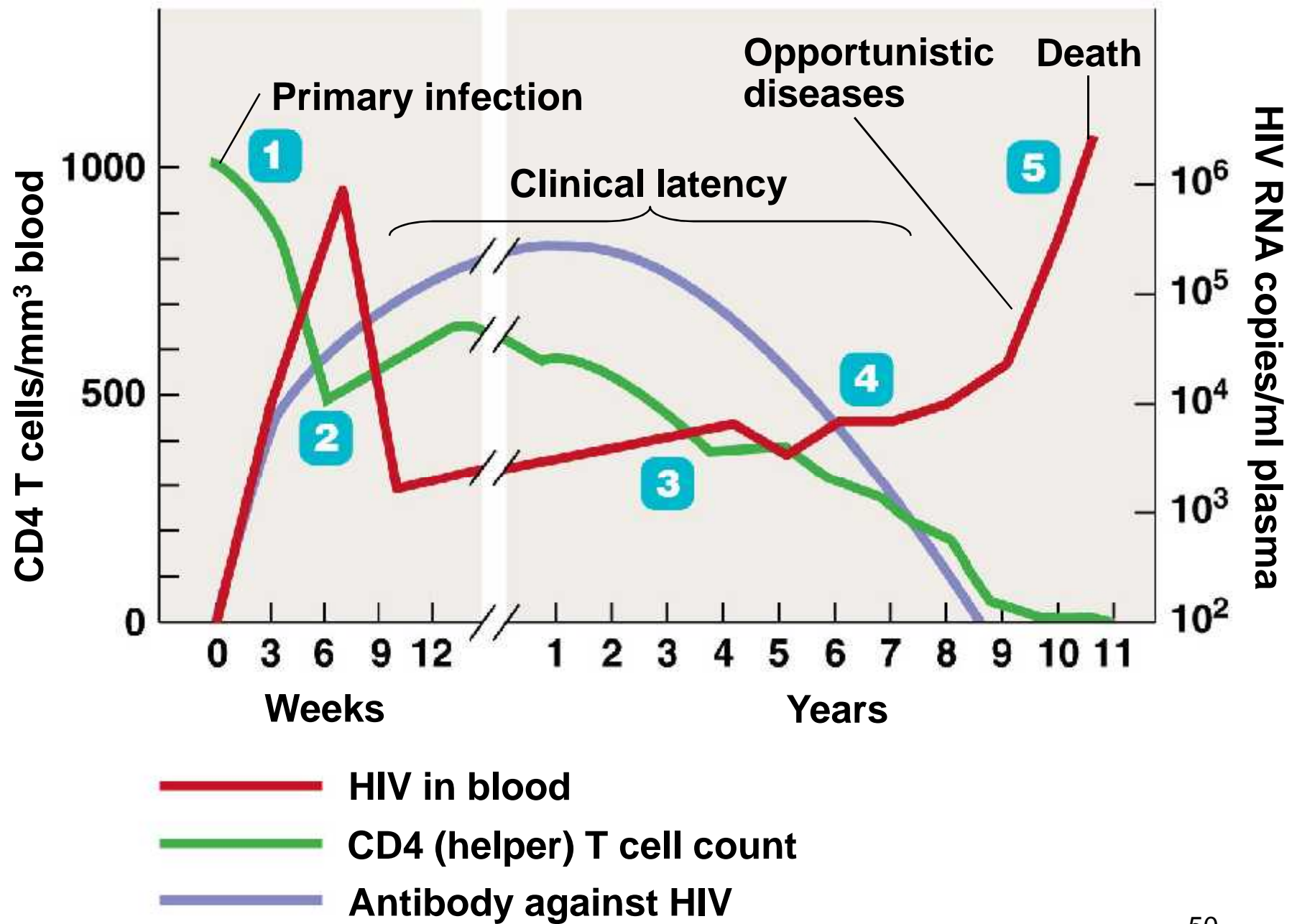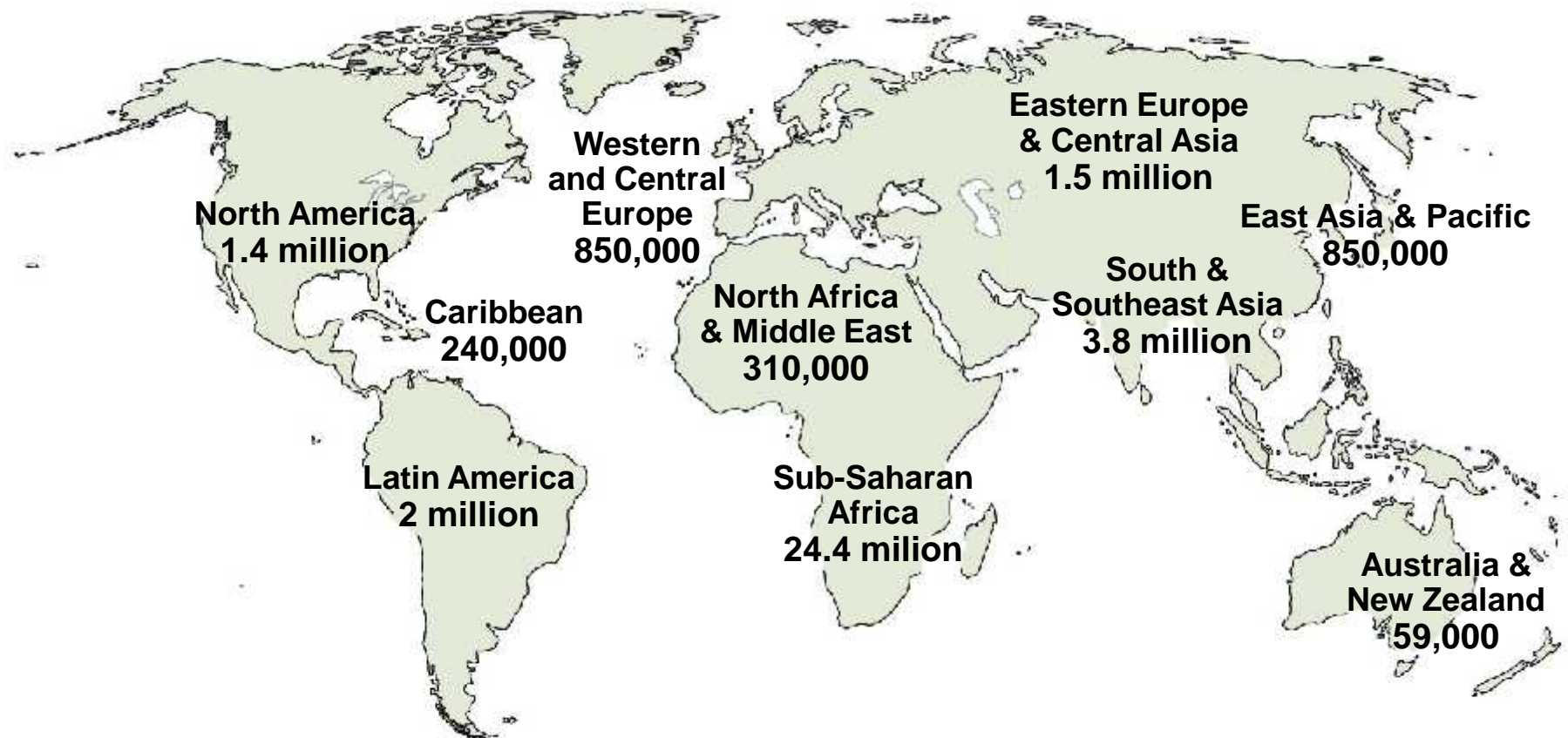TEM 85 nm

(c)

(d)

(e)

(f)

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Figure 18.19 The course of AIDS

Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - Epidemiology of AIDS
    - First recognized in young male homosexuals in the U.S.
    - Now found worldwide
    - HIV in blood, semen, saliva, vaginal secretions, and breast milk concentrated enough to cause infection
      - Must be injected into the body or contact a tear or lesion in the skin or mucous membranes

Figure 18.20 The global distribution of HIV/AIDS

North America
1.4 million

Western and Central Europe
850,000

Eastern Europe & Central Asia
1.5 million

East Asia & Pacific
850,000

Caribbean
240,000

North Africa & Middle East
310,000

South & Southeast Asia
3.8 million

Latin America
2 million

Sub-Saharan Africa
24.4 milion

Australia & New Zealand
59,000
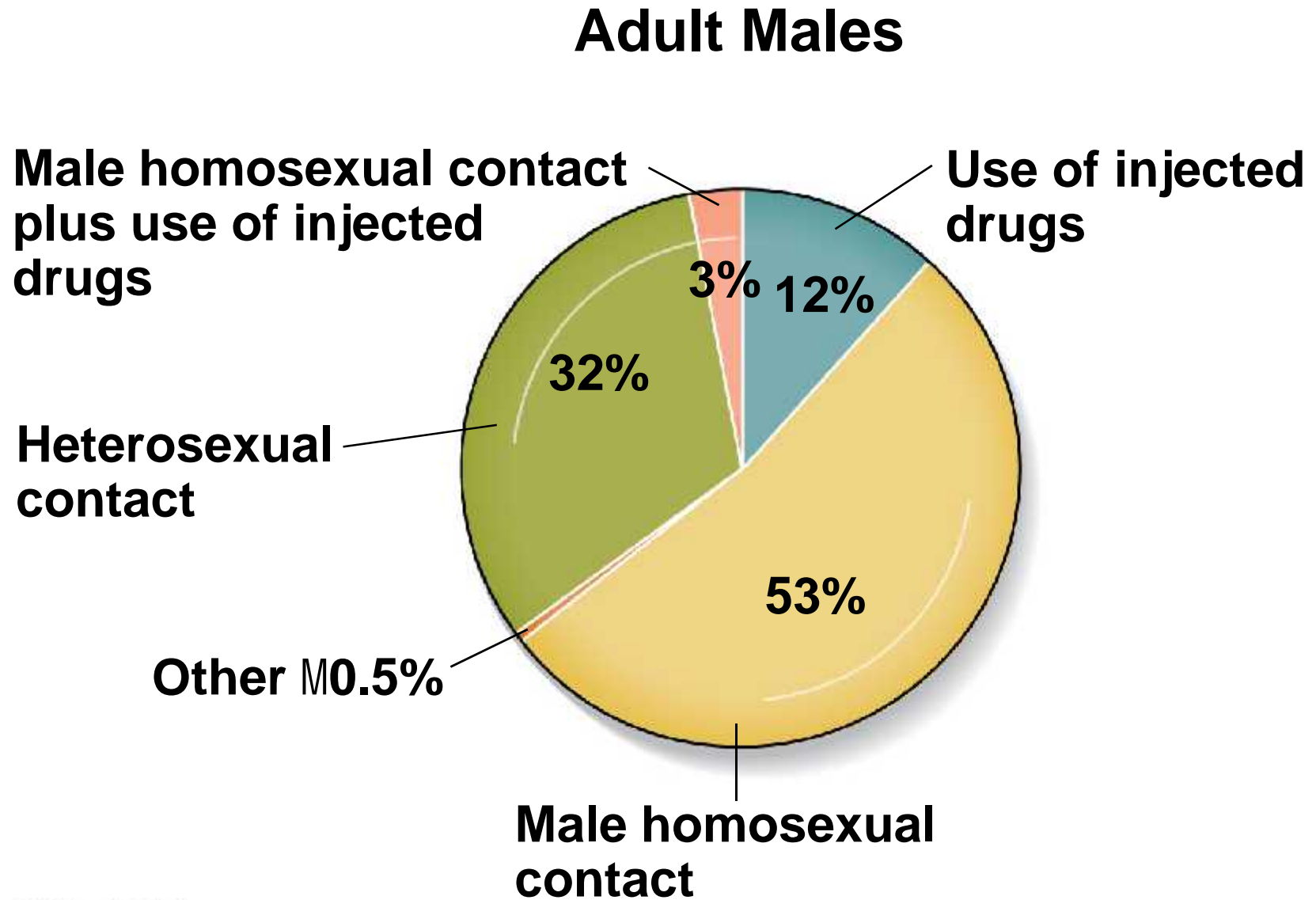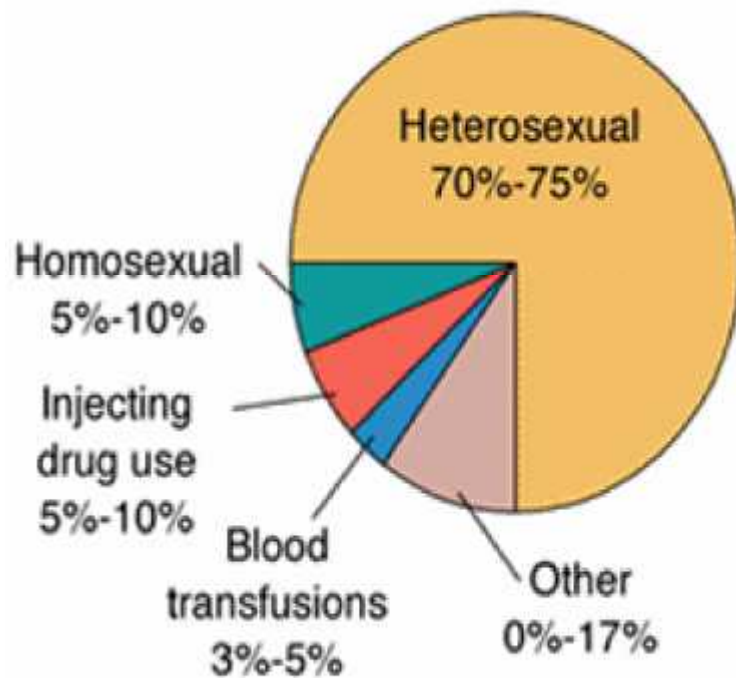
Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - Epidemiology of AIDS
    - HIV is transmitted primarily via sexual contact and intravenous drug use
    - HIV is also transmitted across the placenta and in breast milk
    - Certain behaviors increase the risk of infection
      - Anal intercourse
      - Sexual promiscuity
      - Intravenous drug use
      - Sexual intercourse with anyone engaging in the previous three behaviors

Figure 18.21 Modes of HIV transmission in males over 12 years of age in the U.S. during 2007

Adult Males

Male homosexual contact plus use of injected drugs

Use of injected drugs

3% 12%

32%

Heterosexual contact

53%

Other M 0.5%

Male homosexual contact

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HIV Transmission in United States and Rest of the World

(a) World
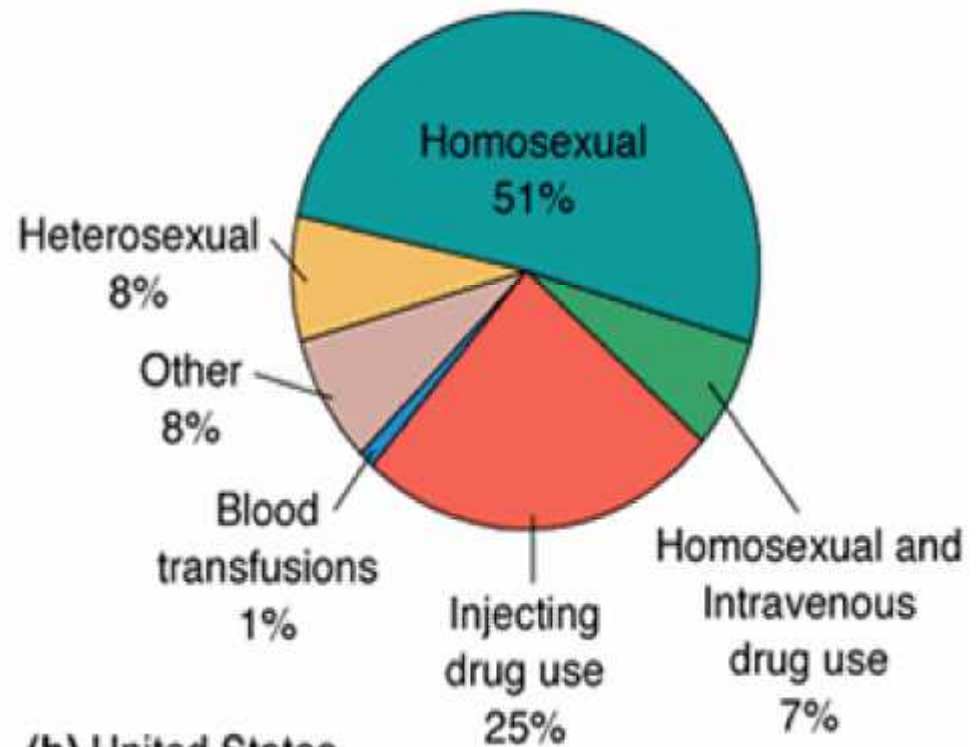
Heterosexual 70%-75%
Homosexual 5%-10%
Injecting drug use 5%-10%
Blood transfusions 3%-5%
Other 0%-17%

(b) United States

Homosexual 51%
Heterosexual 8%
Other 8%
Blood transfusions 1%
Injecting drug use 25%
Homosexual and Intravenous drug use 7%

Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - Diagnosis, treatment, and prevention
    - Diagnosis involves detecting antibodies against HIV
      - Can indicate infection with HIV but not presence of AIDS
    - Small percentage of infected individuals are long-term nonprogressors
      - Appear not to develop AIDS
      - Possibly because of defective virions, mutated coreceptors for the virus, or well-developed immune systems

Immunodeficiency Diseases

- **Acquired Immunodeficiency Diseases**
  - Diagnosis, treatment, and prevention
    - Antiretroviral therapy (ART)
      - A "cocktail" of several antiviral drugs
      - Reduces viral replication, but infection remains
    - Vaccine development has been problematic
    - Diseases associated with AIDS are treated individually
    - Individuals can slow the AIDS epidemic with numerous personal decisions

Coronaviruses
Structure and Composition

- Enveloped
  - Spike proteins resemble solar corona or crown
- 120-160 nm
- Positive-strand RNA (27-32 kb)
- Cytoplasmic replication
- Budding into ER and Golgi
- Notoriously difficult to propagate in culture
- High frequency of recombination
- Cause colds and severe acute respiratory syndrome (SARS)
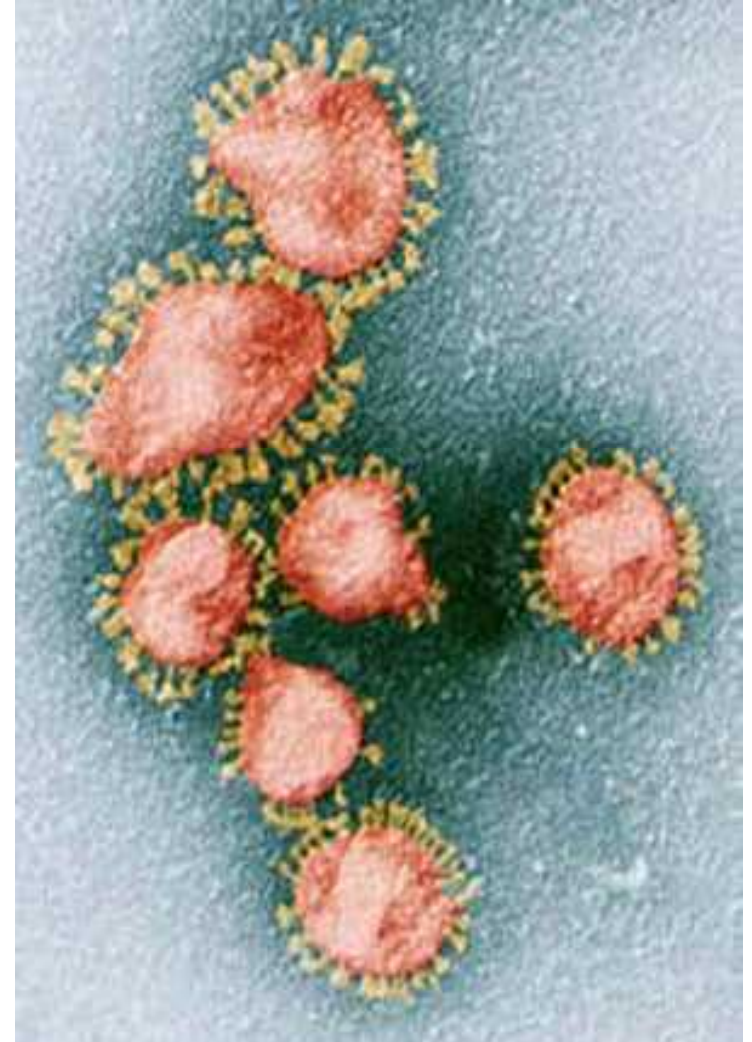
Coronavirus Infections

- Pathogenesis
  - Limited knowledge
  - Highly species-specific
  - Typically mild upper respiratory infections ("colds") that remain localized
    - Exception: SARS
  - Immunity is not durable
    - Many people become resusceptible after a few years
- Laboratory Diagnosis
  - ELISA - may not discriminate past infections
  - HA
  - PCR
  - Virus isolation is difficult (often impossible) and requires great expertise

Severe Acute Respiratory Syndrome

- Initial outbreak in SE Asia
  - Hong Kong and Singapore first reported
  - Disease originated in China
  - Originally thought to be from wild game markets
    - Palm civet cat (which isn't a cat) - *Paradoxurus hermaphroditus*
    - Raccoon dog (which isn't a dog) - *Nyctereutes procyonoides*
  - It is a bat virus
    - Chinese horseshoe bats (*Rhinolophus sinicus*)
    - No virus isolation
      - Amplification of coronavirus RNA from anal swabs
      - Serology
      - It is highly-similar, but not identical to SARS-CoV
        - Mutations have most likely occurred in transmission from bats to civets to humans
        - Reverse genetics of SARS-CoV and some bat viruses has been done
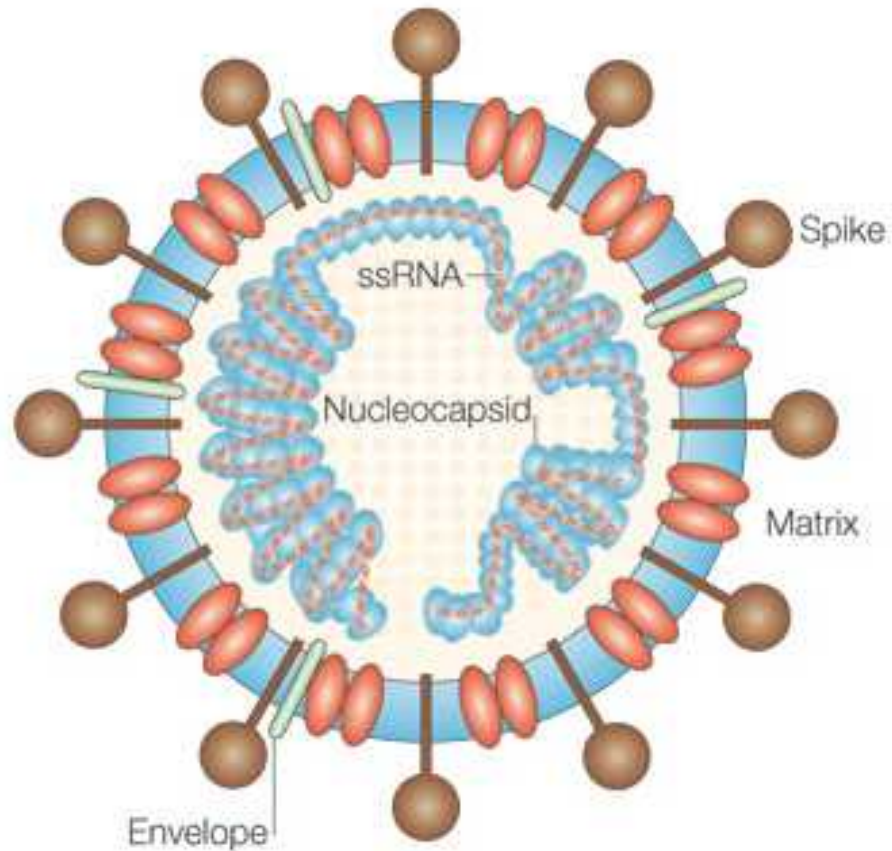 - No animal pathogenesis model

60

SA

a SARS-CoV genome

b SARS-CoV virion

61
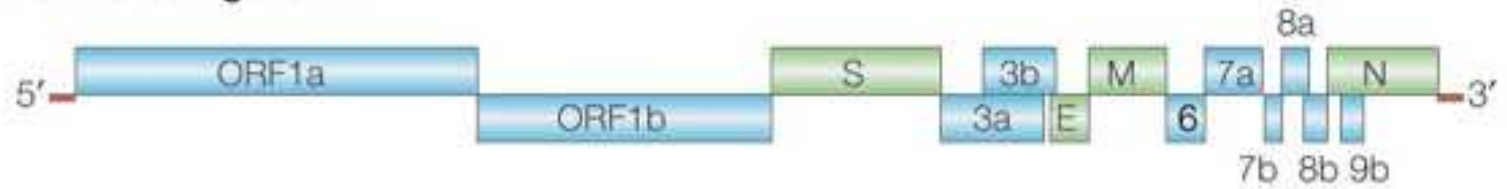
Influenza (The flu)

- Influenza is a disease of the respiratory tract.

- Transmission occurs as a result of inhaling airborne respiratory droplets from an infected individual.

- Infection by the influenza virus results in the destruction of epithelial cells of the respiratory tract, leaving the host open to secondary infections from bacteria such as *Haemophilus influenzae* and *Staphylococcus aureus*.

- It is these secondary infections that are responsible for the great majority of fatalities caused by influenza. Generally, sufferers from influenza recover completely within 10–14 days, but some people, notably the elderly and those with chronic health problems, may develop complications such as pneumonia
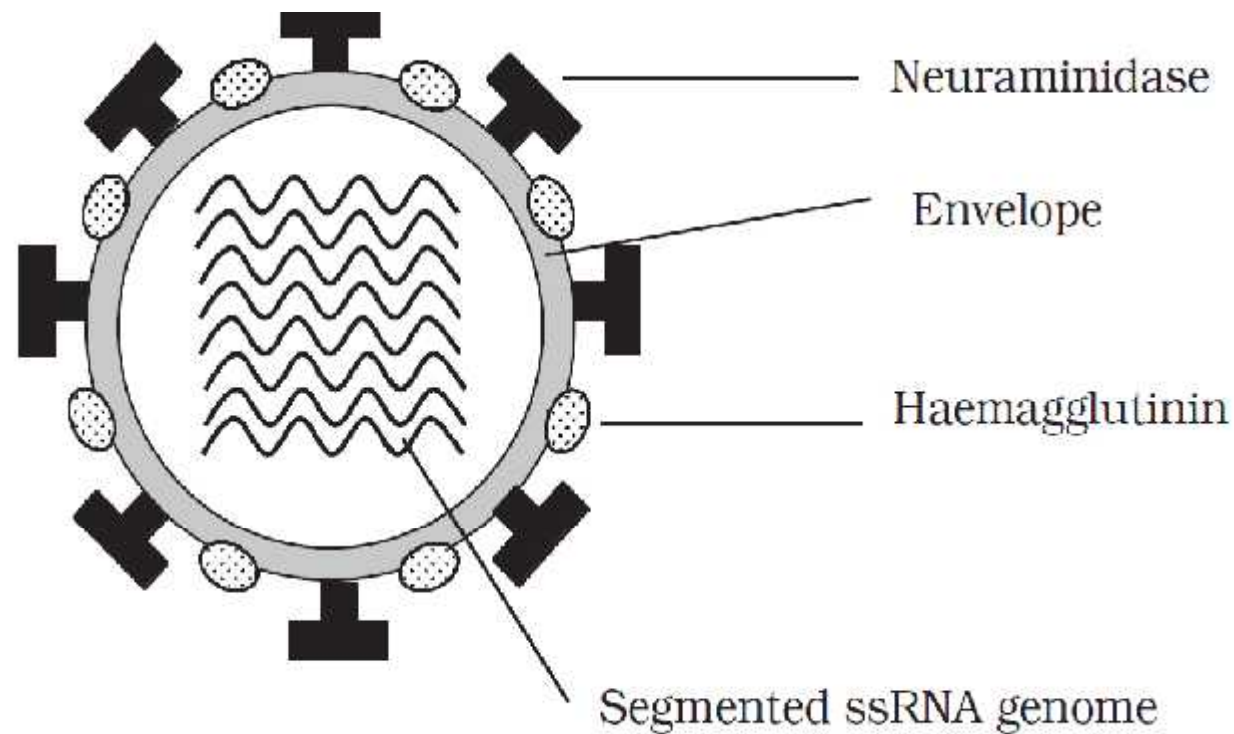
Figure 10.19 The influenza virus. The RNA segments are bound to protein, fo
nucleocapsid, and are surrounded by further protein. The two types of spike assi
attachment and penetration of the virus into its host

- 1. **Neuraminidase** is an enzyme which hydrolyses sialic acid, thereby assisting inthe release of viral particles.

- *2. Haemagglutinin* enables the virus to attach to host cells by binding to epithelialsialic acid residues. It also helps in the fusion of the viral envelope with the cellmembrane.

- Both types of spike act as antigens, proteins that stimulate the production of antibodies in a host.

- One of the reasons that influenza is such a successful virus is that the 'N' and 'H' antigens are prone to undergoing changes (*antigenic shift*) so that the antigenic 'signature' of the virus becomes altered, and host immunity is evaded.

- Different strains of the influenza virus are given a code denoting which variants of the antigens they carry; the strain that caused the 1918 pandemic, for example, was N1H1, while the one responsible for the outbreak of 'bird flu' in SE Asia in 2003/4 was H5N1.

Microbiology for Nursing students

Chapter Eight: Mycology

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Dr. Sulaiman Alnaimat 2015
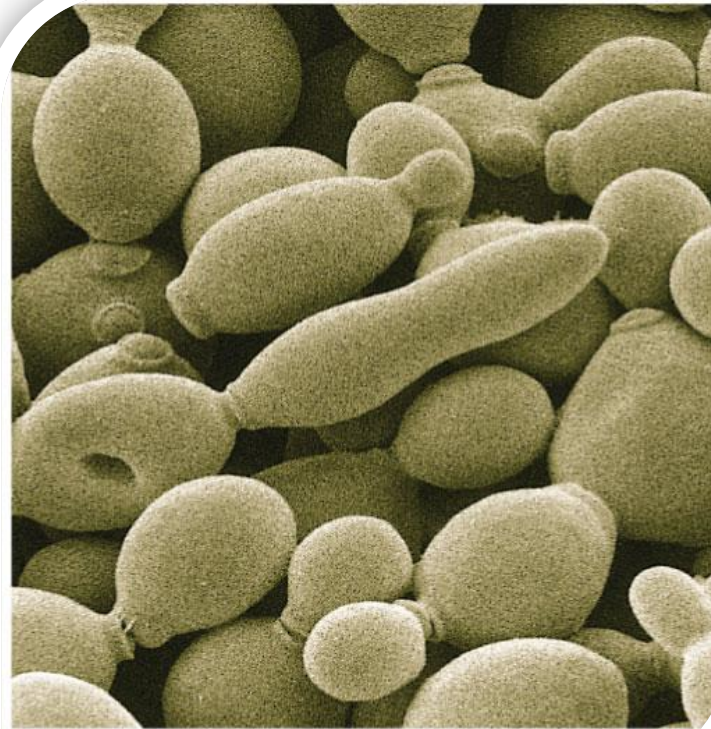
Characteristics of Fungi

Mycology, the science devoted to the study of fungi

• Most of fungi are free-living in nature where they function as **decomposers** in the energy cycle. Of the more than 200,000 known species, fewer than **200** have been reported to produce disease in humans.

• These diseases, the **mycoses**, have unique clinical and microbiologic features and are increasing in **immunocompromised patients**.

Structure

- Have what eukaryotic cells have. (linear chromosomes)
- In fungi, the dominant sterol is **ergosterol** (Target for antifungal drugs); in mammalian cells, it is cholesterol.
- Fungi are usually in the **haploid** state, although diploid nuclei are formed through nuclear fusion in the process of sexual reproduction.
- Cell wall in fungi contains the polysaccharides **chitin** in close association with each other and with **structural proteins**

Table 5.1 Some Differences between Fungi and Bacteria

Properties	Fungi	Bacteria
Nucleus	Eukaryotic; nuclear membrane; more than one chromosome; mitosis	Prokaryotic; no membrane; nucleoid; only one "chromosome"
Cytoplasm	Mitochondria; endoplasmic reticulum; 80S ribosomes	No mitochondria; no endoplasmic reticulum; 70S ribosomes
Cytoplasmic membrane	Sterols (ergosterol)	No sterols
Cell wall	Glucans, mannans, chitin, chitosan	Murein, teichoic acids (Gram-positive), proteins
Metabolism	Heterotrophic; mostly aerobes; no photosynthesis	Heterotrophic; obligate aerobes and anaerobes, facultative anaerobes
Size, mean diameter	Yeast cells: 3–5–10 μm. Molds: indefinable	1–5 μm
Dimorphism	In some species	None

- **The opportunistic fungi** are those found in the environment or in the normal flora that occasionally produce disease, usually in the compromised host.

Dimorphism in pathogenic fungi typically depends on temperature:

At 37ºc: Yeast form.

At 25ºc: Mold form.

5

Reproduction

- Asexual or sexual processes. Reproductive elements produced asexually are termed **conidia**. Those produced sexually are termed **spores**

- Asexual reproduction involves mitotic division of the haploid nucleus and is associated with production by budding spore-like conidia or separation of hyphal elements.

- In sexual reproduction, the haploid nuclei of donor and recipient cells fuse to form a diploid nucleus, which may then divide by classical meiosis.

Fungal morphology

- The size of fungi varies immensely. A single cell without transverse septa may range from bacterial size (2–4 μ) to a macroscopically visible structure.

- Mushrooms (complex organization)

- Initial growth from a single cell may follow either of two courses, **yeast** or **mold** .

Dimorphism

- Some species can grow in **either a yeast or a mold phase**, depending on environmental conditions. These species are known as **dimorphic fungi**.

Fungal morphology

Consists of many hyphae.

Hyphae (Sing: Hypha): Long filaments of cells joined together.

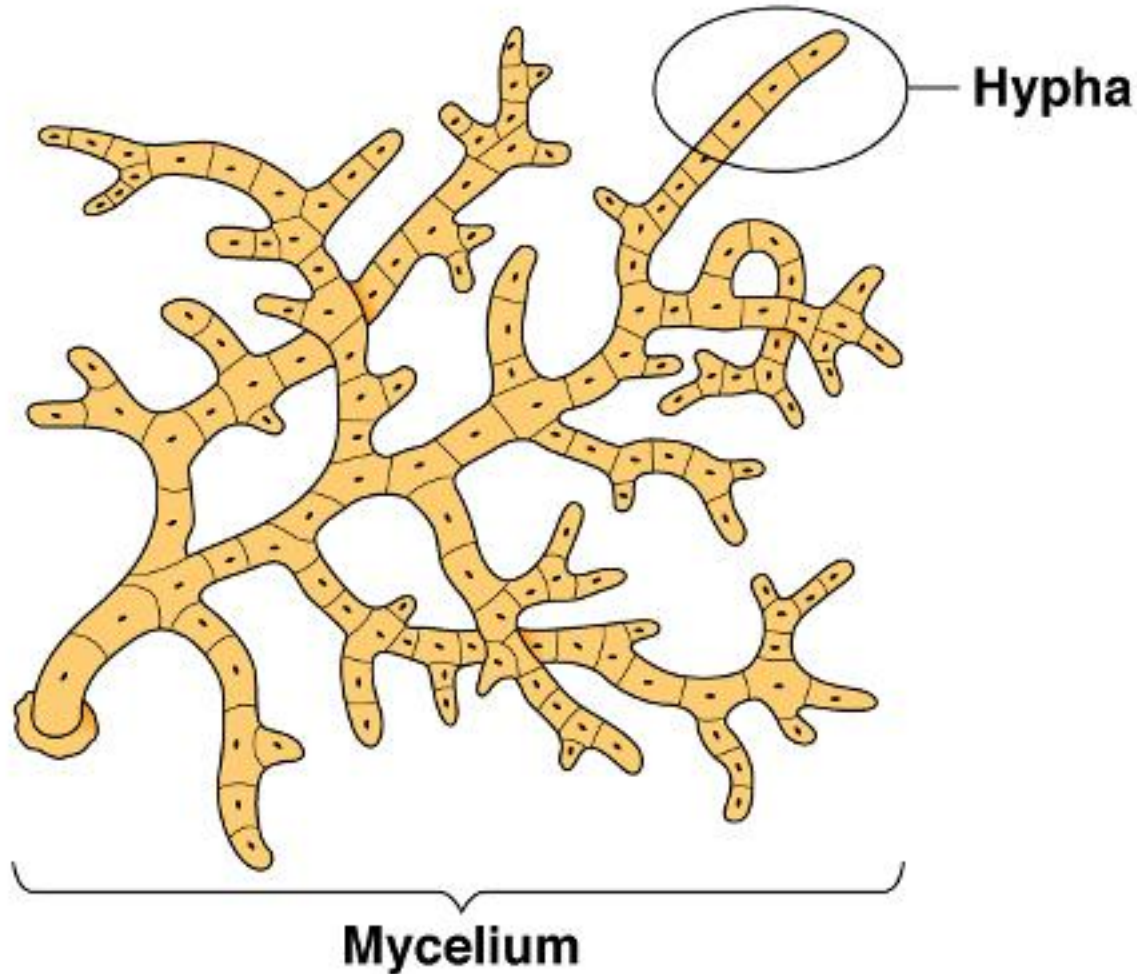 **Septate hyphae:** Cells are divided by cross-walls (septa).

  Coenocytic (Aseptate) hyphae: Long, continuous cells that are not divided by septa.

  Hyphae grow by elongating at the tips.

  Each part of a hypha is capable of growth.

Mycelium: Large, visible, filamentous mass made up of many hyphae

Mycelium: Large, Visible Mass of Hyphae

Hypha

Mycelium

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Mitochondrion
Bud vacuole
Secretory vesicles
Bud
Nucleus
Golgi complex
Pore in nuclear membrane
vacuole
Endoplasmic reticulum
Vacuolar membrane
Lipid granule
Bud scar
Cell membrane
Vacuolar granules
Storage granule
Thread-like mitochondrion
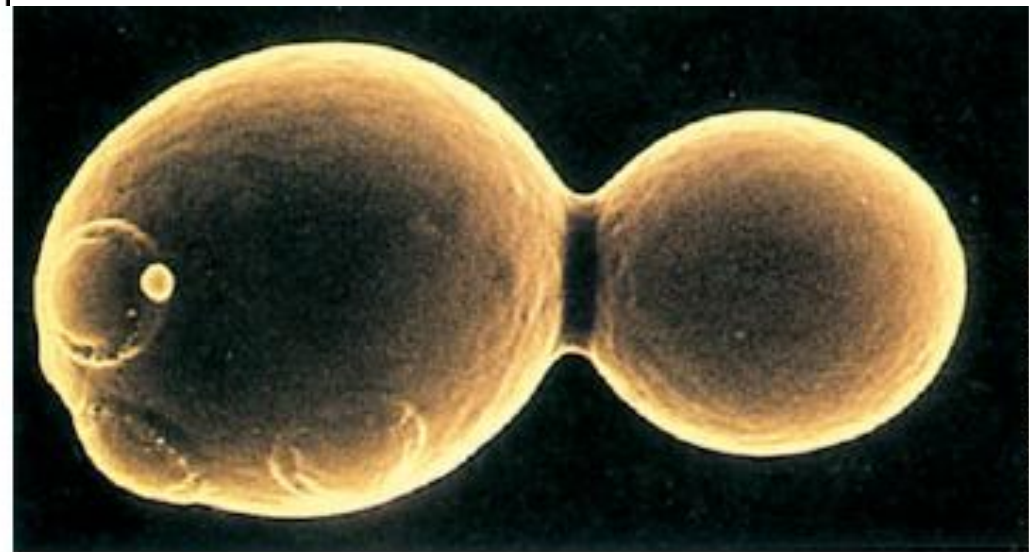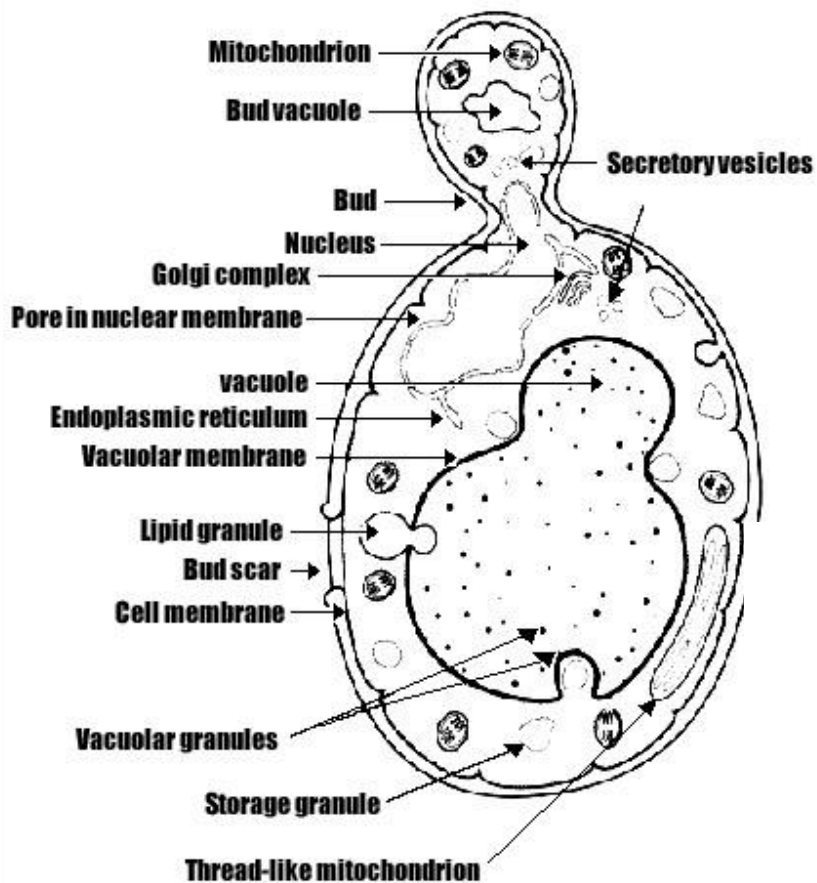
J. Forsdyke/SPL/Photo Researchers

Figure 20.30 Scanning electron micrograph of the common baker's and brewer's yeast *Saccharomyces cerevisiae* **(ascomycetes).** Note the budding division and scars from previous budding. A single large cell is about 6 μm in diameter.

Human diseases caused by fungi are called **mycoses**. The diseases are divided into three groups depending on where they occur on our body. These groups are:

Superficial ▶	These infect the skin, nails and hair.
Subcutaneous ▶	These infect the deep layers of the skin.
Systemic ▶	These are the most severe fungal diseases. An unsuspecting person may inhale the pathogenic fungal spores. Some spores stay in the lungs and grow while others enter the bloodstream, travel around the body and infect other organs.

- Most fungal infections are due to opportunistic pathogens; these affect people who are already ill or have a suppressed immune system (e.g. in patients who have been given an organ transplant, or in AIDS patients).

- In a perfectly healthy person the fungus would not normally cause disease. True pathogens can cause disease in even the healthiest person

- Like bacteria, fungi can produce toxins.

- The most widespread and dangerous of these are the **aflatoxins** produced by the mould called *Aspergillus flavus*. These are carcinogenic, which means they can cause cancer.

SUPERFICIAL FUNGI

Superficial mycoses:

Infections of hair shafts and superficial epidermal cells. Prevalent in tropical climates.

Dermatophytes

- Dermatophytoses are superficial infections of the skin and its appendages, commonly known as ringworm, athlete's foot.

- They are caused by species of the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*, which are collectively known as dermatophytes.

- These fungi are highly adapted to the nonliving, keratinized tissues of nails, hair, and the stratum corneum of the skin. The source of infection may be humans, animals, or the soil.

Microsporum

Courtesy of
The Geraldine Kaminski Medical Mycology Library
Produced by: David Ellis and Roland Hermanis
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Epidermophyton

Courtesy of
The Geraldine Kaminski Medical Mycology Library
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Opportunistic mycoses

Caused by organisms that are generally harmless unless individual has weakened defenses:

AIDS and cancer patients

Individuals treated with broad spectrum antibiotics

Very old or very young individuals (newborns).

Examples:

Aspergillosis: Inhalation of *Aspergillus* spores.

- Yeast Infections or Candidiasis: Caused mainly by *Candida albicans*. Part of normal mouth, esophagus, and vaginal flora. Candidiasis is an endogenous infection

Candida (Soor)

- At least 70% of all human Candida infections are caused by *C. albicans*
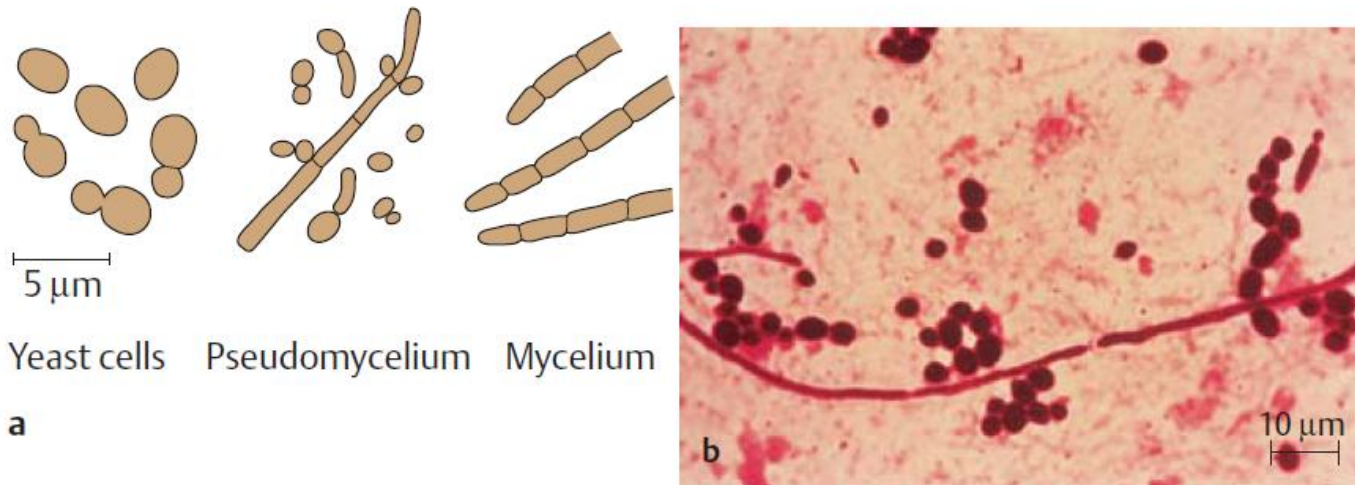

Candida albicans

5 μm

Yeast cells   Pseudomycelium   Mycelium

a

b   10 μm

Fig. 6.2   *Candida albicans.*
a Morphological forms.
b Gram staining of sputum: Gram-positive yeast cells and hyphae.
Clinical diagnosis: candidiasis of the respiratory tract.

Morphology and culture

- Gram staining of primary preparations reveals *C.albican*s to be a Gram-positive, budding, oval yeast with a diameter of approximately 5 µm.

Image Courtesy of M. McGinnis
Copyright © 2000 Doctorfungus Corporation

A) Oral soor; surface infection of cheek mucosa and tongue by *Candida albicans* in an AIDS patient.

B) Chronic mucocutaneous candidiasis in a child with a cellular immunodeficiency syndrome.

Cutaneous mycoses:

Fungal infections of the skin, hair, and nails. Secrete keratinase, an enzyme that degrades keratin. Infection is transmitted by direct contact or contact with infected hair (hair salon) or cells (nail files, shower floors).

Examples:

Ringworm (*Tinea capitis* and *T. corporis*)

Athlete's foot (*Tinea pedis*)

Jock itch (*Tinea cruris*)

- **Tinea corporis (ringworm):** *Microsporum canis* and *Trichophyton mentagrophytes*. Affects hairless skin.

- **Tinea pedis (athlete's foot):** *T. rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum*. Affects mainly the lower legs.
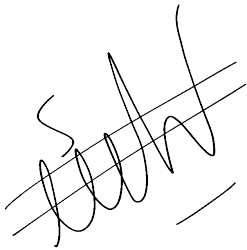
Cutaneous Mycosis

Ringworm skin infection: *Tinea corporis*
Source: Microbiology Perspectives, 1999

Subcutaneous mycoses:

Fungal infections beneath the skin. Caused by saprophytic fungi that live in soil or on vegetation. Infection occurs by implantation of spores or mycelial fragments into a skin wound. Can spread to lymph vessels.

كم كنت سعيدا بكم
اسأل الله لكم التوفيق جميعا
وان يجعلكم من أهل العلم والإيمان وان ينفع بكم الامة

اخوكم : د. سليمان النعيمات